Quattro II
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Instrument Description

Introduction

The Quattro II triple quadrupole mass spectrometer is available with a wide range of inlet systems and ionisation techniques including gas and liquid chromatography (GC & LC), electrospray (ESP), atmospheric pressure chemical ionisation (APCI), electron impact (EI), chemical ionisation (CI), fast atom bombardment (FAB) and thermospray (TSP).

Quattro II utilises two high performance, research grade quadrupole mass analysers, each incorporating a prefilter assembly to protect the main analyser from contaminating deposits. A hexapole collision cell, between the two mass analysers, can be used to induce fragmentation to assist in structural investigations.

Ions emerging from the second mass analyser are detected by the Dynolite™ detector system. A low noise photomultiplier operating typically with a gain of $10^3$ amplifies the ion current collected. A similar detector, situated close to the exit of the first analyser, is used for MS analysis.

A PC-compatible computer runs the MassLynx NT software system to control Quattro II, and to acquire and manipulate data from it.
The Vacuum System

The facing diagram shows the vacuum system as required for a Quattro II fitted with all available options. Many of the components shown are omitted if not relevant to a particular instrument's configuration.

Fine Pumping

Quattro II is equipped with two water cooled turbomolecular pumps, providing independent fine pumping of the analyser and source regions. Details of the operation and maintenance of the pumps can be found in the manufacturer's manuals provided.

Rotary Pumping

Rough pumping, inlet pumping and turbomolecular pump backing is by direct drive rotary pump(s). For API-only systems, a single pump meets all pumping requirements. Additional pumps are fitted for optional techniques as shown in the facing diagram.

Rotary pumps are mounted outside the main bench to reduce the effects of vibration, and for ease of maintenance. Details of the operation and maintenance of the pumps can be found in the manufacturer's manuals provided.

Automatic Control

Except when using the solids introduction probe, routine procedures are performed automatically using remotely controlled vacuum valves.

Pressure Measurement

Depending upon instrument configuration, the pressure in the various regions of the system is monitored by active inverted magnetron gauges and Pirani gauges. Readings can be displayed on the MassLynx NT tune page.

Vacuum Protection

The vacuum system is fully interlocked to provide adequate protection in the event of:

- a fault in the vacuum system
- a failure of the power supply
- a failure of the water supply
- temporary high pressure due to high solvent or sample levels
- vacuum leaks.
Ionisation Techniques

Electrospray

Electrospray mass spectrometry (ESMS) allows very large and very labile molecules to be analysed routinely. The sample, in solution, emerges from a high voltage capillary tube into a strong electrostatic field at atmospheric pressure producing an aerosol of highly charged droplets. Evaporation of solvent from these droplets results in sample ions. In the case of proteins and other biomolecules, such ions may carry several electronic charges.

Electrospray is compatible with on-line HPLC and capillary electrophoresis but is restricted to polar samples.

Atmospheric Pressure Chemical Ionisation

Atmospheric pressure chemical ionisation (APcI) is a soft ionisation technique able to accept the entire eluent from a standard 4.6mm diameter HPLC column.

Sample droplets are vaporised in a heated chamber before emerging into a region of solvent ions formed within the atmospheric pressure source by a corona discharge. Ionisation occurs as a result of chemical reactions between the sample molecules and solvent ions.

The Combined EI/CI Source

Most Quattro II systems are equipped with a combined source capable of operating in both the electron impact (EI) and chemical ionisation (CI) modes. In either mode, the source may be used in conjunction with the reference inlet, the insertion probe, the GC inlets or the particle beam interface.

Electron Impact

Electron impact is the classical ionisation technique in which gas phase sample molecules are ionised in collisions with high energy electrons. The source is heated to ensure sample vaporisation.

Chemical Ionisation

When the combined EI/CI source is operated in the chemical ionisation mode, a reagent gas is admitted to the source at a relatively high pressure. The gas molecules are ionised by the electron beam. Sample ions are generated in reactions with these gas ions. CI is a “softer” ionisation technique than EI, producing less sample fragmentation and generally a stronger molecular ion.

Desorption Chemical Ionisation

Polar samples, liable to decomposition under normal CI conditions, are deposited on the tip of the DCI probe and thermally desorbed into a CI environment.
FAB and Dynamic-FAB

In the static-FAB mode, sample molecules are deposited on the tip of a probe and are ionised by bombardment with fast moving Cs⁺ ions.

Dynamic-FAB utilises a probe which allows a continuous flow of liquid, normally the eluent from an HPLC system, to enter the source. Flow rate limitations with this technique are severe.

Thermospray / Plasmaspray

Thermospray is a widely-used technique for the analysis of polar samples amenable to high pressure liquid chromatography (HPLC). Liquid entering the interface is heated and passed through a small orifice to form an aerosol containing desolvated ions.

When a high voltage is applied to an electrode within the source a plasma containing sample ions is generated. This variation, known as plasmaspray, allows both polar and non-polar samples to be analysed.

The thermospray interface will cope with HPLC flow rates of up to approximately 2 ml/min.
Sample Inlet Systems

With the exception of the vacuum lock, all of the permanent inlets are located on the left hand side of the mass spectrometer. The heated GC transfer line passes through a computer-controlled oven and enters the ion source through a combined inlet flange along with the reference inlet and CI gas.

The optional particle beam interface, when fitted, is located to the right of the source. For all other ionisation techniques, and for solids and involatile liquids, sample is admitted by probe. Apart from the atmospheric pressure techniques (ESP and APcI) the probe is inserted through a vacuum lock.

Electrospray Co-axial and Triaxial Probes

The electrospray probe allows sample, solvent and a stream of nebulising gas (nitrogen), to flow into the electrospray source.

The triaxial flow probe is a direct alternative to the standard co-axial electrospray probe. It incorporates a sheath tube which allows additional solvent to be transported to the probe tip and mixed co-axially with the sample flow immediately before spraying.

The CE Probe

The CE probe is used to interface capillary electrophoresis (CE) to a standard electrospray source. The probe utilises a triaxial flow arrangement whereby the CE eluent is mixed with a suitable make-up solvent at the probe tip, and then nebulised using nitrogen gas.

The APcI Probe

For APcI operation sample, along with the solvent and nebulising gas, is admitted to the mass spectrometer through the heated APcI probe.

The SFC/APcI Interface

The SFC/APcI interface can be configured for both capillary column supercritical fluid chromatography (cSFC) and packed column SFC (pSFC).

The Reference Inlet

The reference inlet is a simple reservoir inlet system for the introduction of volatile liquid reference compounds, such as heptacosa (FC43), for the purpose of calibration. If required this inlet can also be used to introduce volatile liquid samples. Computer controlled solenoid valves control the introduction of the sample into the source and the evacuation of the reservoir.
The CI Reagent Gas Inlet

The CI reagent gas inlet is incorporated in the combined GC inlet flange. A needle valve controls the rate of gas flow into the ion source. The pumping out and admission of gas is controlled remotely by solenoid valves.

The Insertion Lock

The main component of the vacuum lock is a 90 degree rotation ball valve which isolates the system from atmosphere when no probe is inserted. The manually operated pumping valve allows the outer section of the lock to be evacuated prior to probe insertion.

The Solids Insertion Probe

The solids insertion probe is admitted to the ion source through the insertion lock.

Samples are loaded into a replaceable glass holder located in the tip of the probe shaft so that when it is inserted through the lock the sample can be evaporated into the ion source. A powerful heater combined with continuous flow water cooling allows precise computer control of the sample temperature.

The Robot Probe

A modified version of the probe and lock is available, fitted with a pneumatically operated transport mechanism and motorised vacuum valves. This set-up is used in conjunction with a laboratory robot, or with a GC autosampler, to provide fully automated probe sampling under MassLynx NT control.

The DCI Probe

The optional DCI probe is admitted to the ion source through the insertion lock.

Polar samples, liable to decomposition under normal CI conditions, are deposited on the coil at the tip of the DCI probe and thermally desorbed into a CI environment.

The Static-FAB Probe

Sample molecules are deposited directly onto the tip of the probe prior to insertion via the insertion lock.

The Dynamic-FAB Probe

The dynamic-FAB probe allows a continuous flow of liquid, normally the eluent from an HPLC system, to enter the source.
The Direct GC Inlet

The capillary column inlet line consists of stainless steel tubing, the bore of which is large enough to allow fused silica capillary columns to be fed from the GC oven directly into the ion source. The end of the column reaches to within a few millimetres of the ion beam.

Other types of capillary column may be connected to a short length of fused silica capillary, using zero dead-volume connectors. The fused silica passes through the GC inlet into the ion source.

The Jet Separator Inlet

When packed GC columns (or wide bore capillary columns at high flow rates) are to be used, then the column eluent is passed into the ion source via the optional jet separator. The jet separator reduces to acceptable levels the flow of carrier gas into the mass spectrometer while increasing the concentration of sample molecules in the eluent reaching the source.

The Particle Beam LC-MS Interface

By passing the HPLC eluent in turn through a pneumatic nebuliser, a desolvation chamber and a momentum separator to remove solvent, a stream of sample particles is produced.

These particles then enter the standard EI/CI source for analysis. In the case of EI, library-searchable spectra are produced.

The particle beam interface will cope with HPLC flow rates of up to approximately 1 ml/min.

Ion Optics

The Quattro II is a tandem quadrupole mass spectrometer for MS and MS-MS analysis. The principle components of the ion optical system are shown in schematic form below.
The lenses that separate the ion source from MS1 allow ions generated in the source to be focused into the mass analyser. Generally, each ion source has its own set of lens tuning parameters. Analyser tuning parameters, conversely, are constant irrespective of the ionisation source in use (for operation at the same resolution and over the same mass range).

MS1 is a quadrupole mass analyser equipped with two prefilters and a post-filter. The prefilters protect the main rods from contamination, and the first prefilter should be removed periodically for cleaning. The Altrincham lens with its associated detector (intermediate detector or D1) separates MS1 from the collision cell. MS1, operated in conjunction with D1, provides the simplest and most sensitive method of performing conventional MS analysis.

The hexapole collision cell enables ions generated in the ion source and selected for by MS1 to be collisionally activated causing them to fragment. The hexapole collision cell is a RF only device, the RF field within the cell efficiently refocuses ions scattered by the collision induced dissociation process.

MS2 is a quadrupole mass analyser with its own prefilter and post-filter. Its primary purpose is to mass analyse the products of fragmentation reactions occurring in the collision cell.
Collision Induced Dissociation

The ionisation processes occurring within the source of the mass spectrometer can yield a population of sample ions that contain a wide distribution of internal energies.

Ions that possess high internal energies generally undergo one or more fragmentation processes within the ion source. This is typical for ions formed under EI conditions.

Ions of low internal energy will be sufficiently stable to pass from the ion source to the detector without dissociation. This is common for ions formed under soft ionisation processes such as TSP/PSP and API.

Ions of intermediate internal energy, however, may undergo dissociation processes as they pass through the mass spectrometer. These less stable ions, which dissociate in transit due to excess internal energy, are said to be formed by unimolecular dissociation processes.

Alternatively, dissociations may be collisionally induced. In this process a portion of the kinetic energy of the ion is converted to internal energy by colliding with a neutral gas phase species usually in the pressurised collision cell of a MS-MS instrument. Ions that have undergone this collisional excitation process may subsequently fragment. Collision induced dissociation (CID) processes are much more efficient than unimolecular dissociations.

Collisional activation is a valuable method of generating structural information if the primary ionisation process does not impart enough internal energy for spontaneous fragmentations to occur.
When operating in the MS-MS mode, detector 2 is always used. In the MS mode three options are available: MS, Q1F and MS2.

### MS Operating Modes

<table>
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<th>MS1</th>
<th>D1</th>
<th>Collision Cell</th>
<th>MS2</th>
<th>D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>Resolving</td>
<td>On</td>
<td></td>
<td></td>
<td>Off</td>
</tr>
<tr>
<td>Q1F</td>
<td>Resolving</td>
<td>Off</td>
<td>RF Only (Pass all masses)</td>
<td>On</td>
<td></td>
</tr>
<tr>
<td>MS2</td>
<td>RF Only (Pass all masses)</td>
<td>Off</td>
<td>RF Only (Pass all masses)</td>
<td>Resolving</td>
<td>On</td>
</tr>
</tbody>
</table>

The MS mode, where MS1 is used as the mass filter and ions are detected at D1, is the most common and most sensitive method of performing MS analysis. This is directly analogous to using a single quadrupole mass spectrometer.
The Q1F and MS2 modes of operation are not normally used for analytical mass spectrometry. Both these modes, however, provide useful tools for instrument tuning prior to MS-MS analysis and for fault diagnosis.

The MS2 mode of operation may occasionally be used preferentially in circumstances where high pressure source techniques, such as thermospray or electrospray, are in use.

**MS-MS Operating Modes**

The basic features of the four common MS-MS scan functions are summarised below.

<table>
<thead>
<tr>
<th></th>
<th>MS1</th>
<th>Collision Cell</th>
<th>MS2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daughter Ion Spectrum</strong></td>
<td>Static (parent mass only)</td>
<td></td>
<td>Scanning</td>
</tr>
<tr>
<td><strong>Parent Ion Spectrum</strong></td>
<td>Scanning</td>
<td>RF only (pass all masses)</td>
<td>Static (daughter mass only)</td>
</tr>
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<td><strong>Multiple Reaction Monitoring</strong></td>
<td>Static (parent mass only)</td>
<td></td>
<td>Static (daughter mass only)</td>
</tr>
<tr>
<td><strong>Constant Neutral Loss Spectrum</strong></td>
<td>Scanning (synchronised with MS2)</td>
<td></td>
<td>Scanning (synchronised with MS1)</td>
</tr>
</tbody>
</table>
The Daughter Ion Spectrum

This is the most commonly used MS-MS scan mode.

Typical applications:

- Structural elucidation (for example peptide sequencing).
- Method development for MRM screening studies:

  Identification of daughter ions for use in MRM “transitions”.

  Optimisation of CID tuning conditions to maximise the yield of a specific daughter ion destined for use in a MRM transition.

Example:

Daughters of the specific parent at \(m/z\) 502 from heptacosa (FC43) in EI positive ion mode.

The result (measured at the final detector):
The Parent Ion Spectrum

Typical application:

- Structural elucidation.

Complementary/confirmatory information (for daughter scan data).

Example:

Parents of the specific daughter ion at m/z 69 from heptacosa (FC43) in EI positive ion mode.

![Diagram of MS1 and MS2]

MS1: scanning from m/z 65 to 505

Collision Cell: RF only (pass all masses)

MS2: static at m/z 69 (daughter mass only)

The result (measured at the final detector):
MRM: Multiple Reaction Monitoring

This mode is the MS-MS equivalent of SIR (Selected Ion Recording). As both MS1 and MS2 are static, this allows greater “dwell time” on the ions of interest and therefore better sensitivity (~100×) compared to scanning MS-MS.

Typical application:

- Rapid screening of “dirty” samples for known analytes.
  
  Environmental, for example dioxin analysis.
  Forensic / toxicology, for example screening for target drugs in sport.

Example:

Monitor the transition (specific fragmentation reaction) \( m/z \ 184 \rightarrow 102 \) for dibenzo-P-dioxin in EI positive ion GC-MS-MS mode.

The result:

*MRM does not produce a spectrum as only one transition is monitored. As in SIR, a chromatogram is produced.*

<table>
<thead>
<tr>
<th>GC-MRM</th>
<th>GC-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>. High specificity</td>
<td>. Non-specific</td>
</tr>
<tr>
<td>. Good signal / noise</td>
<td>. Poor signal / noise</td>
</tr>
</tbody>
</table>
The Constant Neutral Loss Spectrum

The loss of a specific neutral fragment or functional group from an unspecified parent or parents.

Typical applications:

- Screening mixtures for a specific class of compound that is characterised by a common fragmentation pathway.
  
  For example: drug metabolism studies, screening culture broths for “penicillins” in the free acid form (loss of 159 in EI positive ion mode).

Example:

Screen an unknown organic mixture for the presence of carboxylic acids (having molecular weights in the 144-544 range).

The scans of MS1 and MS2 are synchronised. In this example when MS1 transmits a specific parent ion, MS2 “looks” to see if that parent loses a fragment of mass 44. If it does it will register at the final detector.

Loss of 44 (CO₂) in EI positive ion mode is characteristic of carboxylic acids.

The result:

The “spectrum” will show the masses of all parents that actually lost a fragment of 44 daltons.
MassLynx NT Data System

A PC computer runs the MassLynx NT software system to control Quattro II, and to acquire and manipulate data from it. A high resolution colour monitor is also supplied. Interaction with MassLynx NT is via the mouse and keyboard using menu-driven commands. Printing, file management and other routine procedures are performed using the appropriate Windows modules.

Software

The following software packages are supplied with Quattro II:

- MassLynx NT
- Screen Capture, a utility for copying user selected areas of any Windows display. The selected area can be printed directly, or saved as a bitmap file for importing into other Windows NT applications.
- DataBridge, a utility to convert Lab-Base data files into MassLynx format.
- Microsoft Windows NT graphical environment.
- Mouse configuration.
- MaxEnt maximum entropy data enhancement algorithm (option).
- BioLynx (option).
- Visual Basic (option). This software development system is used to create macros for use with MassLynx NT and other Windows NT applications.

The MassLynx NT User's Guide describes the many facilities of the Micromass software. Documentation for the other software is also supplied.
Routine Procedures

Automatic Pumping and Vacuum Protection

The following diagram illustrates the procedures and automated sequences used to control and protect Quattro II’s vacuum system. The starting point in this diagram is a partial shutdown state in which power is off but water is on.
The status of the system is indicated at all times by the front panel lamps:

<table>
<thead>
<tr>
<th>VENT</th>
<th>PUMP</th>
<th>OPERATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>Red</td>
<td>Green</td>
</tr>
<tr>
<td>Red</td>
<td>Green</td>
<td>Red</td>
</tr>
</tbody>
</table>

- **Pump Rough**
  - Flash

- **Pump High (Standby)**
  - Green

- **Operate**
  - Green
  - Green

- **Transient Pressure Trip (Operate)**
  - Red
  - Flash

- **Transient Pressure Trip (Standby)**
  - Red

- **RF Trip**
  - Red

- **Turbo Wind Down**
  - Flash

- **Turbo Shutdown**
  - Red

- **Venting**
  - Flash

- **Vented**
  - Green

**Protection**

**Transient Pressure Trip**

The transient trip is designed to protect the instrument from potentially damaging pressure surges and operates routinely when solvent elutes from the GC column.

Should the vacuum gauge(s) detect a pressure surge above the preset trip level (normally set at $10^{-4}$ mbar by software) the following events occur:
• The system turns off the critical source, analyser and detector voltages.

• The green OPERATE and PUMP lamps are extinguished, and the red lamps show.

• Acquisition continues though, of course, no real data are recorded.

When the vacuum recovers, voltages are restored and PUMP and OPERATE are green.

_The period during which the trip was operative will appear in a raw total ion chromatogram as a period of reduced baseline noise._

Further deterioration of the system pressures results in a “vacuum fault” condition and the system is shut down (see below).

**Water Failure**

A failure of the water supply for more than a brief period results in a “Vacuum Fault” condition and the system is shut down (see below).

**Vacuum Fault**

A vacuum fault can occur as a result of:

• a failure of the water supply.

• a vacuum leak.

• a malfunction of the turbomolecular or rotary pumps.

A vacuum fault causes the turbos to stop pumping and is signalled by the red PUMP lamp flashing as the turbos wind down. This lamp becomes steady when the turbos stop. Proceed as follows:

Switch off the power to the rotary pumps.

Investigate and rectify the cause of the fault (see “Fault Finding”).

Pump down the instrument as described earlier in this chapter.

**Power Failure**

In the event of an unexpected failure of the electrical supply the instrument is vented safely. If power is unlikely to be restored quickly, follow the shutdown procedure described later in this chapter. When power is restored follow the start-up procedure.

• Should the power fail and then be restored while the instrument is unattended, the system will advance to the “Select PUMP” position in the left hand column of the flow diagram earlier in this chapter.
Start Up Following a Complete Shutdown

Preparation

If the instrument has been unused for a lengthy period of time, proceed as follows:

Check the level of oil in each rotary pump sight glass. Refill or replenish as necessary as described in the pump manufacturers literature.

Turn on the cooling water and check that a reasonable flow is established. Inspect all hose connectors for leaks.

Ensure that an ion source is correctly fitted (see the relevant chapters of this manual) and, as appropriate, check that:

- the vacuum lock ball valve is closed.
- the GC transfer line is blanked off.
- the thermospray inlet is blanked off.
- the particle beam nebuliser is removed, with a rubber bung in its place.

**Warning:** Ensure that the drying gas exhaust (electrospray and APcI systems) and the rotary pump exhaust are separately vented to atmosphere external to the laboratory via fume cupboards or cold traps.

Switch on the power to the mass spectrometer.

At the rear of the instrument switch on in turn:

- the main circuit breaker.
- the **ELECTRONICS** switch.
- the **PC** switch.
- all other switches.

Follow the on-screen instructions and, when requested, enter the Windows NT password. For further information, consult the Windows NT manual.

*As supplied, Windows NT is automatically activated during the start-up sequence whenever the computer is switched on.*

After a few seconds, the Windows NT program manager (or, if **Minimise on Use** has been selected, its icon) is displayed. The MassLynx icon is located in the MassLynx group window.
Using the left mouse button, double click on MassLynx.


Allow several seconds for MassLynx to load.

From the MassLynx top line, select **Acquire**, followed by **Control Panel**, to display the acquisition control panel.

Select **Instrument**, followed by **Tune Mass Spectrometer**, to select the tune window.
Pumping

Select Other, followed by Pump, to initiate the pumping sequence.

Open the gas ballast valves on all rotary pumps.

Rotary pumps are normally noticeably louder when running under gas ballast.

When the rotary pumps have run under gas ballast for about 1 hour:

Close the gas ballast valves.

When system pressures have reached acceptable levels, the red Pump lamp is replaced by green.

Using the Instrument

Quattro II is now almost ready to use. To complete the start up procedure and to prepare for running samples, follow the instructions in the following sections of this chapter.
Start Up Following Overnight Shutdown

Preparation

On the instrument’s front panel, check that the green PUMP lamp is illuminated.

From the MassLynx top line, select Acquire, followed by Control Panel, to display the acquisition control panel.

Select Instrument, followed by Tune Mass Spectrometer, to select the tune window.

Select Window and Pressures to display the vacuum gauge readouts. Check that all vacuum levels are adequate.

Select the appropriate ionisation mode using the Ion Mode command.

Referring to the relevant chapter(s) of this manual, check that:

- the source and inlet for the technique to be used are correctly fitted.
- all gas, sample and electrical connections are made.
- flow rates and temperatures are set to their operating levels.
- the normal Aperture setting (EI/CI source) is selected.

Operate

Quattro II can only enter the OPERATE mode and acquire sample data when:

- vacuum levels are adequate.
- Quattro II recognises that the correct source is fitted.

To select the OPERATE mode:

Click on OPERATE on the MassLynx tune page.

_The analyser and detector high voltage supplies are on. The green OPERATE lamp illuminates._

The system is now ready to accept samples and acquire data.
Tuning and Calibration

Before sample data are acquired the instrument should be tuned and, for the highest accuracy, calibrated using a suitable reference substance.

Tuning Source and MS1

Consult the relevant section of this manual for information concerning source tuning procedures in the chosen mode of operation.

Adjust the tuning parameters in the **Source** and **MS1** menus to optimise peak shape and intensity at unit mass resolution.

Tuning MS2

Set up a tune page display as shown.

Adjust the tuning parameters in the **MS2** menu to optimise for peak shape and intensity at unit mass resolution.

Return to the **MS1** menu and, with **Function** set to **MS2**, adjust the values of **Lens 5** and **Lens 6** to maximise transmission to the final detector.

In the **MS2** menu care should be taken to optimise the value of the collision energy. Note that **Collision** and **Lens 9** are interactive parameters.
Calibration

Information concerning the calibration of Quattro II is provided in “Acquiring Data” in the MassLynx NT User’s Guide and in the separate publication “Quattro II Guide to Mass Calibration”.

Data Acquisition

The mechanics of the acquisition of sample data are comprehensively described in “Acquiring Data” in the MassLynx NT User’s Guide. Refer to that publication for full details.

Data Processing

The processing of sample data is comprehensively described in the MassLynx NT User’s Guide. Refer to that publication for full details.
Setting Up for MS-MS Operation

The following notes provide a worked example for the acquisition of daughter ion data. The experiment is performed in the EI positive mode using heptacosa (FC43) as a model analyte. Heptacosa, admitted from the reference inlet, provides a stable and persistent source of ions for instrument tuning in both the MS and MS-MS modes of operation.

The basic tuning and optimisation principle demonstrated below (with heptacosa) may be applied in other ionisation modes simply by substituting an appropriate persistent source of ions. For example, in the FAB mode, caesium iodide or glycerol matrix peaks may be used. In thermospray or API, solvent background peaks may be used.

The basic sequence of events is as follows:

- Tuning MS1.
- Tuning MS2.
- Parent ion selection.
- Fragmentation.

Tuning MS1 and MS2

These two procedures have been described earlier in this chapter.

Parent Ion Selection

For maximum sensitivity in daughter ion analysis the centroid of the parent ion selected by MS1 must be accurately found.

The nominal mass of the parent is first determined (if unknown) by viewing it at the first detector (MS1):

Set up a 1 box display in the tune page and set Function to MS. Observe the candidate parent ion in the tune display and determine its nominal mass.

*In this example the heptacosa ion at m/z 219 will be used as a model parent.*
The accurate top of the parent ion can be found experimentally by performing a “daughter ion scan” over a restricted mass range in the absence of collision gas.

Set up a peak display similar to that shown.

Place the mouse cursor on the **Set** mass for peak 2 and type in the nominal mass of the parent ion selected by MS1, in this case 219.

Zoom in on peak 2.

Select **Other** followed by **Scope Parameters**. Set **Mass Increment** to 0.1 m/z.

Using the left and right arrow controls, vary the set **Mass** value between 218.5 and 219.5 while observing the intensity of the non-fragmented parent ion (transmitted through MS1 and MS2) in the tune display.

Adjust the **Set** mass in this manner to optimise the intensity of the parent.
Fragmentation

Set up a wide range daughter ion scan by adjusting the Mass and Span parameters for peak 2.

At this point, with the collision gas off, a few daughter ions of low intensity may be visible, as in the following example. These are the products of unimolecular dissociations.

Argon (99.995% pure), or a 3:1 mixture of argon (99.995% pure) with xenon (99.993% pure), is recommended as the collision gas.

Select Gas and turn on Collision.

Adjust the needle valve on the front panel to admit sufficient gas to attenuate the parent ion peak by about 50%.

Adjust the Collision and Lens 9 parameters in the MS2 menu to produce the desired degree of fragmentation. (These two parameters are interactive in MS-MS operation.)
In daughter ion analysis maximum transmission (sensitivity) can be achieved by adjustment of the following parameters:

- Increasing **Repeller** on the **Source** tune window.
- Increasing **IEnergy 1** on the **MS1** window.
- Optimising **Collision** (**MS2**).
- Optimising **Lens 9** (**MS2**).
- Optimising **Lens 5** and **Lens 6** (**MS1**).
- Optimising collision gas pressure (needle valve).

Additionally, transmission can be improved at the expense of specificity by reducing **HM Res** on the **MS1** window. In most cases, where chemical interference with the parent ion is not acute, the loss of specificity is negligible.
Changing Between Ionisation Modes

Venting

Quattro II should be prepared for venting in the following way:

Withdraw the probe.

Call up the tune page.

From the **Gas** menu ensure that all options are deselected.

Put the instrument into standby by deselecting **OPERATE**.

**Caution:** Always close gas ballast valves before venting the instrument.

The system can now be vented.

On the tune page select **Other** followed by **Vent**.

![Instrument Under Vacuum]

Select **OK** at the warning message.

The system now starts its automatic venting sequence.

Allow the system to vent automatically.

*After the vacuum pumps are switched off, a few minutes will elapse until the automatic vent valves open. Shortly after this, the system will come up to atmospheric pressure and the source can be removed.*

**Caution:** Venting the system by opening the vacuum lock valve is not recommended. Forcing the system to vent in this way can cause debris to be sucked into the analyser with a consequent loss of performance.
Removing a Non-API Ion Source

Withdraw the GC column (if fitted).

Disconnect the LC transfer line (if fitted).

Unplug the source connectors.

Disconnect all gas supply and pumping lines to the source.

Undo the two black thumb screws.

Pull the source out of the instrument.

Place the source on its stand and secure with the thumb screws provided.

Fitting a Non-API Ion Source

Fitting of the non-API ion source is the reverse of the above removal procedure. If the source is to be fitted following a session of API operation it is, of course, necessary first to remove the API source as described in the following pages.

Ensure that the RF lens and adapter flange are removed.

Ensure that the differential aperture and the correct guide rods are fitted.
Fitting the API Source

Having vented the system and removed the non-API ion source as described above, proceed as follows:

Unscrew and remove the two source guide rods. Note the location of the tapped holes from which the rods are unscrewed.

Withdraw the differential aperture from the analyser entrance orifice.

Identify two tapped holes above those from which the guide rods were removed.

Screw the API guide rods into these upper holes.

The API guide rods are shorter and narrower than the non-API rods.
Using the two socket headed bolts, fit the adapter flange to the analyser housing. Ensure that the O ring is correctly located on the analyser side of the flange.

Connect the angled Fisher plug to the socket on the inside of the housing.

Connect the two short black RF lens leads to the two brass feedthroughs below the Fisher socket.

With the inner end of the RF lens assembly resting on the guide rods, connect the four trailing leads to the pins on the lens assembly.

**Caution:** The skimmer is, of necessity, a delicate precision component requiring careful handling at all times.

The two leads for the skimmer lens are polarised and are not compatible with the two RF connections, which are interchangeable. It is therefore not possible to connect these four leads incorrectly.
Carefully slide the RF lens assembly along the guide rods until the inner end is felt to engage in the analyser entrance orifice.

Release the quick release clamp and remove the blanking flange. Retain the O ring and clamp.

Insert the API source housing into the analyser housing and secure with the two black thumb nuts.

Using the O ring and quick release clamp, connect the pumping tube to the port on the side of the source.

Check that the manual vent valve is closed.
Removing the API Source

Vent the system as described earlier in this chapter.

Unplug the source connectors.

Open the manual vent valve. Close the valve after a few seconds.

Disconnect all gas supply lines to the source.

The API gas lines are released by depressing the coloured collars on the bulkhead connectors while pulling the tube.

Follow in reverse order the above instructions for fitting the API source.
Pumping Down

For API operation check that the source is correctly configured for the chosen mode of operation. (See “Changing Between Electrospray and APcI” later in this chapter.)

For non-API operation, insert the chosen ion source into the mass spectrometer.

Connect the GC column (if required).

Connect the LC transfer line (if required).

Connect the source connector plugs. (Failure to do so will cause MassLynx not to recognise the ion source in the mass spectrometer.)

Connect all gas supply and pumping lines to the source.

*The API gas lines are connected simply by pushing the tubes into the coloured collars on the bulkhead connectors.*
On the appropriate tune page select **Other** followed by **Pump**.

When vacuum has been achieved, select **Operate** and re-tune the instrument.

**Selecting the Ionisation Mode**

The ionisation mode to be used for tuning and acquisition is set using the **Ion Mode** command on the MassLynx tune page. MassLynx recognises the installed source and will not allow an invalid mode to be selected.

If **Save On Exit** (on the **File** menu) is enabled, previously selected tuning parameters will be restored automatically whenever a new mode is selected.

The solenoid valves which control API gas distribution are operated automatically when the ion mode is selected.
Changing Between Electrospray and APcI

Changing between the two modes of API operation requires removal of the inner source in order to fit the appropriate counter electrode components. This can be achieved without venting the system.

The counter electrode comprises two finely-machined metal blocks, each with four holes inclined to the centre line. When assembled the holes align to form four non-line-of-sight angled pathways.

The counter electrode is sometimes referred to as the “high voltage lens” or the “pepper pot”.

For early instruments, the blanking plug should be fitted for electrospray while the discharge needle is essential for APcI operation.

Later instruments are equipped with separate complete assemblies for each mode.

Removing the Inner Source

Release the two screws [item 1] and remove the end flange [2].

If appropriate, disconnect the sample cone voltage supply lead (purple) from the terminal pin [3]. Some sources are equipped with a spring loaded contact, and item 3 is absent.

Remove the two plastic-headed thumb screws [4] and withdraw the inner source assembly (complete with gas tubes, supply cable and O ring) from the stainless steel housing [5] within the source housing.

Warning: The inner source is heavy and may be hot. Handle with care, using the cable bracket and HT socket to hold the assembly during removal.
Removing the Counter Electrode

Remove the inner source as described above.

Remove the two locating screws [6] to release the counter electrode [7].

If the source is configured for APcI operation, disconnect the gold MRAC socket [8] as the lens is withdrawn.

For early instruments:

Separate the two component parts of the counter electrode, which are precisely aligned by a dowel and spigot.

Separate the APcI discharge needle (complete with insulator block) or the blanking plug from the lens.

*Do not pull the electrical lead to release the discharge needle, a leverage hole is provided in the insulator block to help free the pin if it is a tight fit.*

Reassembling and Replacing the Inner Source

Reassemble and refit the inner source, using the appropriate counter electrode components for the technique to be used, following in reverse order the above instructions.
Shutdown Procedures

Emergency Shutdown

- Switch off the power at the wall mounted isolation switch.

  *Any data not previously saved will be lost.*

- Turn off the water supply.

- Turn off all gases.

Overnight Shutdown

When the instrument is to be unused for any length of time, for example overnight or at weekends, proceed as follows:

Deselect to enter the “standby” mode.

Save any required data.

Depending upon the instrument configuration:

For EI, set **Source Temperature** to 250 and **Aperture** to 0.

Disconnect the water and electrical connections to the solids probe.

Set the GC oven temperature to a low value.

If in the API mode:

  - Turn off the nitrogen supply at the cylinder.

  - For electrospray, reduce the HPLC pump(s) flow rate(s) to 5 µl/min.

  - Withdraw the probe.

Turn off the HPLC pumps (except for electrospray, see above).

Turn off the helium supply to the particle beam nebuliser.

*The rough pumping systems for thermospray and particle beam configurations utilise a high temperature “hot oil” single stage rotary pump. Pumps of this type run at relatively high temperatures, thus preventing water and eluent vapours from condensing in the pump. Hot oil rotary pumps, once started, should not be turned off except for maintenance or when the instrument is to be left unattended for lengthy periods.*
Complete Shutdown

If the instrument is to be left unattended for extended periods, proceed as follows:

Deselect "OPERATE".

Turn off the HPLC pump(s).

Withdraw and disconnect any probe, and store it in a clean secure place.

On the tune window, select Other followed by Vent.

At the prompt confirm the venting procedure.

Turn off all gases at their source.

Save any required data and exit MassLynx (see the MassLynx User’s Guide).

Close any other Windows applications and exit Windows.

At the rear of the instrument switch off in turn:

- the ELECTRONICS switch.

- all other switches.

- the main circuit breaker.

Switch off the power at the wall mounted isolation switch.

Turn off the water supply.

If the instrument is likely to remain unused for about one month, or longer:

Refer to the manufacturer’s literature and drain the oil from the rotary pumps.
Electrospray

Introduction

The electrospray ionisation technique allows rapid, accurate and sensitive analysis of a wide range of analytes from low molecular weight (less than 200 Da) polar compounds to biopolymers larger than 100 kDa.

Generally, compounds of less than 1000 Da produce singly charged protonated molecular ions (M+H+) in positive ion mode. Likewise, these low molecular weight analytes yield (M-H-) ions in negative ion mode, although this is dependent upon compound structure.

The electrospray source can be tuned to induce ion fragmentation in the rotary-pumped region between the sample cone and the skimmer. This can provide valuable structural information for low molecular weight analytes.

High mass biopolymers, for example peptides, proteins and oligonucleotides, produce a series of multiply charged ions. The acquired data can be transformed by the data system to give a molecular weight profile of the biopolymer.
The most common methods of delivering sample to the electrospray source are:

- **Syringe pump and injection valve:**
  
  A flow of mobile phase solvent passes through a Rheodyne injection valve to the electrospray source. This is continuous until the pump syringes empty and need to be refilled. Sample is introduced through the valve injection loop (usually 10 or 20µl capacity) switching the sample plug into the mobile phase flow. Tuning and acquisition are carried out as the sample plug enters the source. (At a flow rate of 10 µl/min a 20µl injection lasts 2 minutes.)

- **Reciprocating pump and injection valve:**
  
  A flow of mobile phase solvent passes through a Rheodyne injection valve to the electrospray source. Sample injection and analysis procedure is the same as for the syringe pump. The pump reservoirs are simply topped up for continuous operation. The most suitable reciprocating pumps for this purpose are those which are specified to deliver a flow between 1 µl/min and 1 ml/min. A constant flow at such rates is more important than the actual flow rate. The injection valve on reciprocating pumps may be replaced by an autosampler for unattended, overnight operation.

- **Infusion pump:**
  
  The pump syringe is filled with sample in solution. The infusion pump then delivers the contents of the syringe to the source at a constant flow rate. This arrangement allows optimisation and analysis while the sample flows to the source at typically 5-30 µl/min. Further samples require the syringe to be removed, washed, refilled with the next sample, and replumbed.

A 50:50 mixture of acetonitrile and water is a suitable mobile phase for the syringe and reciprocating pumped systems. This is appropriate for positive and negative ion operation.

Positive ion operation may be enhanced by 0.1 to 1% formic acid in the sample solution.

Negative ion operation may be enhanced by 0.1 to 1% ammonia in the sample solution. Acid should not be added in this mode.

These additives should be kept out of the mobile phase for flow injection analysis (FIA) studies, to allow easy change over between positive and negative ion analysis.

Degassed solvents are recommended for the syringe and reciprocating pumps. Degassing can be achieved by sonification or helium sparging. The solvents should be filtered, and stored under cover at all times.
It is wise periodically to check the flow rate from the solvent delivery system. This can be carried out by filling a syringe barrel or a graduated glass capillary with the liquid emerging from the probe tip, and timing a known volume, say 10µl. Once the rate has been measured and set, a note should be made of the back pressure readout on the pump, as fluctuation of this reading can indicate problems with the solvent flow.

**Post-column Splitting**

For LC pump flow rates exceeding the recommended maximum of 50 µl/min, a splitting arrangement is necessary. The post-column split consists of a zero dead-volume tee piece connected as shown.

![Diagram of post-column splitting](image)

The split ratio is adjusted by increasing or decreasing the back pressure created by the waste line, by changing either the length or the diameter of the waste tube. A UV cell may also be incorporated in the waste line, avoiding the requirement for in-line, low volume “Z cells”. As the back pressure is varied, the flow rate at the probe tip should be checked as described above.

These principles apply to splitting for both megaflow and normal flow electrospray.
Megaflow

Megaflow electrospray enables flow rates from 200 µl/min to 1 ml/min to be accommodated. This allows microbore (2.1mm) or 4.6mm diameter columns to be interfaced without splitting.

Changing Between Flow Modes

When changing between the two flow modes, it is essential that the correct tubing is used to connect the probe to the sample injector. For megaflow operation 1/16” o.d., 0.007” i.d. peek tubing, easily identified by its yellow stripe, is used. This replaces the standard microbore peek or fused silica tube, together with the ptfle sleeve and 1/16” peek end fitting.
The Triaxial Flow Probe

The triaxial flow probe incorporates a sheath tube which allows additional solvent to be transported to the probe tip and mixed coaxially with the sample flow immediately before spraying. Always use the red peek tubing supplied with the probe to deliver the make-up solvent.

**Caution:** Alternative tubing may affect the voltage on the probe tip, resulting in poor spray stability.

**Warning:** Under no circumstances should metal capillaries be used.

The peek transfer capillary connects to the stainless steel sample capillary in the peek union fixed in the back of the probe housing. As supplied, the sample capillary is cut square and completely deburred.

Cut the peek capillary straight using a sharp scalpel.

*If fused silica is used instead of the the peek capillary, use a GC column cutter to ensure a straight cut.*

Take care to ensure the ends of the transfer capillary and the stainless steel capillary butt against each other in the peek union.

*A poor connection will give rise to dead volumes, resulting in a decrease in sensitivity and, with LC-MS, a loss of chromatographic resolution.*

The CE Probe

The CE probe is used to interface capillary electrophoresis (CE) to a standard electrospray source using a 375µm fused silica column. The probe utilises a triaxial flow arrangement whereby the CE eluent is mixed with a suitable make-up solvent at the probe tip, and then nebulised using nitrogen gas.

The CE capillary extends fully to the probe tip through the stainless steel sheath capillary which carries the make-up solvent. Around the sheath capillary is the nebuliser capillary through which the nitrogen gas flows to the probe tip.

The make-up solvent performs two functions:

- To supplement the CE flow to a level adequate for electrospray operation.
- To make electrical contact between the CE buffer and the probe tip.
Operation

Obtaining an Ion Beam

The source must be assembled and installed as described in “Routine Maintenance”, and the instrument pumped down as described in “Routine Procedures”. The analyser pressure should be in the region of $5 \times 10^{-5}$ mbar.

Connect a supply of nitrogen to the gas inlet at the rear of the instrument. Set the supply pressure to 7 bar (100 psi).

At this stage the instrument gas controls should be off. Front panel flow meters should not register.

Ensure that degassed solvent is flowing through the electrospray probe at a pulse free flow rate of approximately 10 µl/min.

Within MassLynx call up the tune page (see the MassLynx User’s Guide). Specify either Electrospray + or Electrospray - for the Ion Mode.

Connect the nebulising gas line to the probe (red line connector).

Select Gas and turn on ESI Gas 1

Manually adjust the nebulising gas flow from the front panel gauge to deliver approximately 10 litres/hour. In the case of the coaxial probe, adjust the flow to its maximum value, which is limited by the probe tip to around 10-20 litres/hour.

This action will flush out any solvent which may have entered the gas line by capillary action.

Adjust the ESI drying gas flow to approximately 250 litres/hour.

Check the capillary tip of the probe to ensure there is no significant leak of nitrogen escaping past the sides of the tip.

The entire flow of nebulising gas should be flowing directly through the probe tip, maximising the efficiency of nebulisation.

Set the probe stop bar to extend about 5mm from the probe front end.

While holding out the spring-loaded probe securing pin, insert the probe into the source so that the microswitch actuating pin enters the probe.
Push the probe into the source until the stop bar prevents further insertion.
Release the securing pin.

Attach the high voltage and probe cables to the probe.

Select **OPERATE**.

Initially the Source, MS1 and MS2 tune pages, described in the MassLynx NT User’s Guide, should be used for setting the source and analyser operating parameters. The values shown below in “Source Voltages” should allow an ion beam to be obtained in the positive ion mode.

Fine tuning, which follows the initial set-up, is discussed below in “Tuning and Optimisation”.

Beam stability and initial sensitivity can be optimised using the m/z = 42 protonated acetonitrile solvent peak in electrospray positive ion mode (assuming a 50:50 solution of CH₃CN:H₂O, with 0.1 to 1% formic acid, is flowing into the source at 10 µl/min) or any other suitable solvent peak.

Electrospray negative ion tuning and optimisation should be performed on sodium dodecyl sulphate (5 ng/µl), horse heart myoglobin (20 pmol/µl + 0.3% ammonia) or the sample under investigation.
Source Voltages

The following illustration shows the various components of Quattro II’s ion optical system. The name in the table’s first column is the name used throughout this manual to describe the component. The second column shows the term used in the current MassLynx release.

The voltages shown are typical for an instrument in good condition. The polarities given are those actually applied to the electrodes. Only positive values need be entered via the tune page.

<table>
<thead>
<tr>
<th>Component</th>
<th>Tune Page Name</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrospray Probe</td>
<td>Capillary (kV)</td>
<td>+3.5</td>
<td>-3.0</td>
</tr>
<tr>
<td>Counter Electrode</td>
<td>HV Lens (kV)</td>
<td>+0.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>Sample Cone*</td>
<td>Cone (V)</td>
<td>+25</td>
<td>-25</td>
</tr>
<tr>
<td>Skimmer Lens</td>
<td>Skimmer Lens Offset (V)</td>
<td>This lens optimises at a set voltage (typically 5V) higher than the sample cone. Skimmer Lens Offset is set from the Other menu on the tune page.</td>
<td></td>
</tr>
<tr>
<td>Skimmer</td>
<td>Skimmer (V)</td>
<td>+1.5</td>
<td>-1.5</td>
</tr>
<tr>
<td>RF Lens</td>
<td>RF Lens (V)</td>
<td>+0.2</td>
<td>-0.2</td>
</tr>
<tr>
<td>Differential Aperture</td>
<td>Not applicable</td>
<td>Ground</td>
<td></td>
</tr>
<tr>
<td>Prefilter</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The sample cone voltage is sample dependent. The values given are typical for low molecular weight singly charged ions. For tryptic peptides the sample cone optimises at 30 to 40V. Higher sample cone voltages may be required for proteins. In-source fragmentation may be induced by increasing the sample cone voltage (typically 100V). The sample cone voltage may be ramped automatically with m/z to achieve optimum sensitivity across the m/z scale.

CE Buffers and Make-up Solvents

The use of involatile CE buffers such as borate and phosphate are not recommended since they crystallise in the source and can cause blockages and charging. However, low concentrations (mM) may be tolerable for short periods. Generally more volatile buffer salts, such as ammonium acetate, are used. Additionally, buffer additives that give a strong electrospray signal should be avoided, as this can compromise the sensitivity of the sample.

The choice of make-up solvent is somewhat dependent on the samples being analysed by CE. A mixture of 50/50 methanol/water with 1% formic or acetic acid is generally suitable for positive ion work whilst 80/20 IPA/water is more suitable for negative ion work. Acetonitrile should **not** be used as a make-up solvent since it softens the polyamide coating on the CE capillary.
Tuning and Optimisation

Probe Position

The probe position will have an effect on the intensity and stability of the ion beam and, once optimised, should be set by adjusting the probe stop bar.

Setting Up the Probe

Coaxial Probe

The two piece tip of the electrospray probe can have an influence on the intensity and stability of the ion beam. Satisfactory electrospray performance should be obtained with the inner stainless steel capillary protruding about 0.5mm from the outer capillary, as shown.

If the probe tip is adjusted ensure that the tip lock nut is tightened onto the gas sealing O ring so that nebulising gas does not exit from the sides of the tip arrangement.
**Triaxial Probe**

Optimisation of the triaxial-flow probe tip is similar to that for a co-axial probe. Best results are achieved if the sample tube (inner capillary) protrudes 0.1mm from the sheath tube, measured using a scaled magnifying glass.

Fix the sample capillary in the peek connector.

Move the make-up flow tee accordingly.

Position the outer probe tip so that 0.5mm of sheath capillary protrudes.

Tighten the lock nut onto the O ring to prevent the escape of nebulising gas.

**CE Probe**

A continuous length of CE capillary is required to connect the probe tip to the CE instrument with adequate flexibility, including approximately 350mm within the probe.

Cut a length of ptfte tube long enough to shield the CE capillary between the CE instrument and the back of the probe. Slide it over the exposed capillary.

Remove the plastic safety cover from the probe body.
Undo the nuts holding the stainless steel sheath capillary in place and slide it towards the probe tip until the end inside the probe body is exposed.

Feed the CE capillary through the finger tight fitting and into the first tee piece. Guide the capillary carefully into the sheath capillary and feed it forwards.

Relocate the sheath capillary in the tee piece and, with approximately 0.5mm exposed from the probe tip, tighten the nuts to secure it.

Adjust the probe tip to set the correct position. Ensure that the lock nut is tight on the O ring to prevent nebulising gas from escaping.

Feed the CE capillary out of the tip, trim the end square then wipe the end of the capillary with a tissue soaked in solvent.

Retract the capillary so that when the securing nut on the tee piece is tightened, approximately 0.2mm extends beyond the sheath capillary.

Tighten the finger-tight fitting onto the ptfe tube to hold the CE capillary in place.

Connect the ptfe nebulising gas tube to Quattro II’s outlet.

Connect the peek make-up flow tube to a suitable LC pump.

**Warning:** It is essential that the probe earth lead (green) is connected to a suitable earthing point and that the LC pump is earthed.

Check for leaks by setting a make-up liquid flow of 50 µl/min.

Replace the plastic cover.

*Attention must be paid to the probe tip to ensure stable ES operation, good mixing and electrical contact between the make-up solvent and the CE buffer.*

Using a magnifying glass, look end on at the probe tip to check concentricity of the three tubes, applying gentle pressure to the probe tip capillary if needed.

Ensure that both the inlet and outlet ends of the CE capillary are at the same height during operation to prevent any siphoning of buffer occurring.
Sample Cone Position

The position of the sample cone in relation to the skimmer lens and skimmer affects ion beam intensity. The position is adjusted using the four thumb screws on the exterior of the source housing.

The analyser and backing pressures can be seen to vary with the sample cone position. After tuning these usually read respectively $5 \times 10^{-5}$ mbar and $4 \times 10^{-1}$ mbar. These readings should remain steady during routine operation.

An improvement in the vacuum reading may be a result of blockage or partial blockage of a source skimmer aperture. See “Fault Finding”.

Operating Parameters

The following parameters, after initial tuning on the solvent peak, should be optimised using a sample representative of the analytes to be studied.

It will usually be found that, with the exception of the sample cone and skimmer lens voltages, these parameters vary little from the values suggested in the previous section “Obtaining an Ion Beam”.

Drying Gas Flow

It is suggested that the drying gas flow is maintained at approximately 250 litres/hour for liquid flow rates through the probe up to 50 µl/min. Additional drying gas may be required for higher flow rates.

ESI Nebulising Gas Flow

For the co-axial probe, the ESI nebulising gas flow should be set to its maximum.

For the triaxial probe, the ESI nebulising gas flow should be set initially to 20 litres/hour, then adjusted for maximum beam intensity.

Probe (Capillary) Voltage

The electrospray probe (capillary) voltage usually optimises at 3.5 kV although the occasional sample may require tuning of this value between 3.0 and 4.5 kV for positive ion electrospray. For negative ion operation a lower voltage is often necessary, typically between 2.0 and 3.5 kV.
**Counter Electrode (HV Lens) Voltage**

The counter electrode (HV lens) voltage optimises in the range 0 kV to 1.0 kV for both positive ion and negative ion electrospray.

**Sample Cone Voltage**

A sample cone voltage between 25V and 70V will produce ions for most samples, although solvent ions prefer the lower end and proteins the higher end of this range. Whenever sample size and time permit, the cone voltage should be optimised for every sample, within the range 25V-130V. Generally with low molecular weight samples, a higher cone voltage induces fragmentation yielding structural information.

**Skimmer and RF Lens**

These parameters need not be adjusted during day to day operation. Values of 1.5 and 0.2 respectively are typical and, once set, ought not to vary significantly.

**Skimmer Lens Voltage**

A change in sample cone voltage should be accompanied by a re-optimisation of the skimmer lens voltage. The two parameters are interrelated, with the skimmer lens voltage typically 5V higher than the sample cone voltage. The voltage is set using the **Skimmer Lens Offset** parameter, which is accessed via **Other** on the tune page.

**Low Mass Resolution and High Mass Resolution**

Peak width is affected by the values of low mass resolution (**LM Res**) and high mass resolution (**HM Res**). Both values should be set low (typically 5.0) at the outset of tuning and only increased for appropriate resolution after all other tuning parameters have been optimised. A value of 15 (arbitrary units) will usually give unit mass resolution on a singly charged peak up to m/z 1500.

**Ion Energy**

The ion energy parameter usually optimises in the range -5V to +5V. It is recommended that the value is kept as low (or negative) as possible without reducing the height intensity of the peak. This will help obtain optimum resolution.

> If a negative ion energy value of less than -6V can be used without reducing the peak intensity then a full source clean is recommended.

**Source Temperature**

The source temperature is set at 60°C for a 50:50 CH$_3$CN:H$_2$O solvent system. If more than 60% water is present, or flow rates above 50 µl/min are being employed, then a temperature up to 180°C may be required for optimum performance.
Megaflow Hints

With this high flow rate option the set-up procedure involves minimising cluster ions above m/z 200. In the following negative ion spectra from a 50:50 mixture of acetonitrile and water, the upper trace shows significant solvent clusters. These are largely absent in the lower spectrum, achieved by increasing the drying gas flow from 200 to 450 litres/hour.

• Increase the source temperature.

*Temperatures above 100°C are required at flow rates approaching 1 ml/min.*

*Higher temperatures are needed for mobile phases with a high water content.*

*At mobile phase flows approaching 1 ml/min and high drying gas flows the source temperature may not reach 200°C*

• Optimise the drying gas flow.

*The flow meter may read off scale.*

• Check the nebulising gas.

*The nitrogen pressure should be set to 100 psi.*

Check that the flow is not restricted by over tightened ferrules in the probe.

Check that the nebulising gas is not leaking out before reaching the probe tip.

• It is not necessary to change any source voltages.
• Optimise the sample cone position for sensitivity on sample (not solvent) peaks.

Even though the sample cone position may be relatively uncritical as far as sensitivity is concerned, avoid operating in the on-axis position. This will reduce the occurrence of low mass “spikes” and so increase signal to noise.

• For health and safety reasons always ensure the nitrogen exhaust line is vented outside the building or to a fume hood.

There should be a plastic bottle connected in the exhaust line to collect any condensed solvent.

The following positive ion (upper) and negative ion spectra demonstrate typical solvent peaks obtained with a 50:50 mixture of acetonitrile and water flowing at 300 µl/min.
Tuning for CE Operation

Set the make-up flow to 10 µl/min.

Switch the bath and nebuliser gases on.

*The flow rates should be in the range 50 - 150 l/hr and 30 - 40 l/hr respectively.*

Insert the probe and tune initially on the solvent peaks.

To tune on the sample of interest, initially flush the sample through the capillary using a pressure rinse. However, final tuning should be done with the CE voltage applied to the capillary, noting the following points:

- The sample may need to be dissolved in the CE buffer.
- With a voltage applied, signals are usually smaller than with the pressure rinse.
- The probe tip potential may need to be varied to obtain a stable beam.
- The counter electrode (HV lens) normally optimises at zero volts.
- Probe position and nebulising gas flow rate are usually critical for a stable signal.
- The probe may need to be around 15mm back from the fully in position.
- The nebulising gas flow rate should not be set above the maximum travel of the ball in the flow meter as this can draw material through the CE capillary.

Removing the Probe

To remove the probe from the source proceed as follows:

On the tune page deselect Operate.

Disconnect the high voltage and probe cables from the probe.

Pull out the probe securing pin and withdraw the probe.

Switch off the liquid flow.

Select Gas and turn off ESI Gas 1 (or whatever the legend has been changed to).

*As electrospray is an atmospheric pressure technique, no vacuum lock is required.*
Sample Analysis and Calibration

General Information

Care should be taken to ensure that samples are fully dissolved in a suitable solvent. Any particulates must be filtered to avoid blockage of the transfer line or the probe’s capillary. A centrifuge can often be used to separate solid particles from the sample.

There is usually no benefit in using concentrations greater than 20 pmol/µl for biopolymers or 10 ng/µl for low molecular weight compounds.

Higher concentrations will not usually improve analytical performance. Conversely, lower concentrations often yield better electrospray results. Higher levels require more frequent source cleaning and risk blocking the transfer capillary.

Samples with phosphate buffers and high levels of salts should be avoided.

To gain experience in sample analysis, it is advisable to start with the qualitative analysis of known standards. A good example of a high molecular weight sample is horse heart myoglobin (molecular weight 16951.48) which produces a series of multiply charged ions that can be used to calibrate the $m/z$ scale from 800-1700 in either positive ion or negative ion mode.

Polyethylene glycol mixtures, for example 300/600/1000, are low molecular weight samples suitable for calibrating the $m/z$ scale from approximately 100 to 1200 in positive ion mode. A mixture of sugars covers the same range in negative ion mode.

Alternatively, a mixture of NaI and CsI can be used for calibration.

Typical Positive Ion Samples

- Peptides and proteins.
- Small polar compounds.
- Drugs and their metabolites.
- Environmental contaminants (e.g. pesticides/pollutants).
- Dye compounds.
- Some organometallics.
- Small saccharides.

Typical Negative Ion Samples

- Some proteins.
- Some saccharides and polysaccharides.
- Some drug metabolites (e.g. glucuronide conjugates).
- Oligonucleotides.
Inject 10 µl of horse heart myoglobin solution¹ into the solvent flow via the injection valve, with Quattro II set up for positive ion electrospray operation.

*The sample will arrive in the ion source approximately 2 minutes later, depending on the precise flow rate being used and the length of transfer capillary.*

The tuning parameters can be optimised when the sample ions appear, either by setting the tune editor’s scan function to cover the range $m/z$ 800 to 1600, or by choosing two myoglobin ions (say 943 and 998) and scanning a span of 10 amu over each of them.

**Cone** and **Skimmer Lens Offset** will optimise respectively at around 30V and 7V (corresponding to a skimmer lens voltage of 37). The resolution should be set so that the peak width is approximately 1.5 amu at half height.

An MCA acquisition can be initiated from the tune window. Set a scan time of 10 seconds over the $m/z$ range 700-1700 with an interscan delay of 0.1 seconds.

After specifying an appropriate filename and text, **OK** will initiate acquisition.

If the run duration is set to 0 min, the acquisition will continue until **Stop** is selected.
A further injection of sample may be necessary if a flow injection analysis system is being employed. Ensure that the spectrum is acquired from the middle of the 20 pmol/µl loop of sample.

The accumulating spectrum can be observed on the MCA display. Ten scans will be sufficient to produce a spectrum suitable for calibration purposes. To halt an acquisition click on the red STOP tile on the acquisition control panel.

At this stage the data can be evaluated (through Spectrum) and transformed to determine the protein molecular weight (see the MassLynx User’s Guide). If the spectrum is to be used for calibration purposes follow the procedure given in the MassLynx User’s Guide.

¹ Dissolving 2 mg of horse heart myoglobin in 1 ml of water gives 120 pmol/µl stock solution. Dilute this stock solution by a factor 24 with 50:50 CH₃CN:H₂O, 0.5% formic acid to produce a 5 pmol/µl concentration.
A solution of PEGs in a 50:50 solution of CH$_3$CN:H$_2$O, with 1 mmol ammonium acetate$^2$, will yield a spectrum similar to that shown (unit mass resolution set and MCA data acquired over the range $m/z$ 100 to 1200 for approximately 1 min).

The spectrum is obtained from the polymer mass distribution of the various oligomers. Predominantly singly charged ions are observed ($\{\text{M+H}\}^+$, $\{\text{M+NH}_4\}^+$ and, if the sample has been stored in glass, $\{\text{M+Na}\}^+$), and the “spectral pattern” should therefor not be confused with the charge distribution spectrum encountered with myoglobin. It is not appropriate to transform a PEG spectrum.

For calibration purposes follow the same procedure as outlined for myoglobin calibration, using the reference file PEGNH$_4$.

$^2$ Dissolve PEG 300 (10 mg) in 26 ml, PEG 600 (10 mg) in 13 ml, PEG 1000 (30 mg) in 12 ml and PEG 1500 (10 mg) in 1.7 ml using a water solvent. Equal volumes should be mixed together and then a 10× dilution with 50:50 CH$_3$CN:2 mmol aqueous CH$_3$COONH$_4$ prepared as the calibration sample.
Sugar Mixture

A mixture of corn syrup, raffinose, maltose and maltotetraose (500, 20, 100 and 20 ng/µl respectively)³ can be used for ES- calibration over the range m/z 100 to 1500. After conversion to peak top data, a calibration can be made using the SUGNEG reference file.

³ Dissolving corn syrup (1 mg) in 0.5 ml, maltose (1 mg) in 5 ml, raffinose (1 mg) in 12 ml and maltotetraose (1 mg) in 12 ml of 50% aqueous acetonitrile, and then combining equal volumes of these four solutions will produce a mixture at the appropriate concentration.
Sodium Iodide and Caesium Iodide Mixture

Sodium iodide may be used as a calibrant for low molecular weight compounds. It should not be used for accurate protein measurements above 4000 molecular mass. In these cases the multiply charged ion series from a suitable protein (for example ubiquitin, myoglobin or trypsinogen) should be used.

Sodium iodide has the advantage over PPG or PEG as a calibrant in that both sodium and iodine are monoisotopic, hence the calibration peaks are monoisotopic and a given calibration is not compromised when low resolution is used.

A concentration of 2 µg/µl of NaI in 50/50 2-propanol/water serves for both positive and negative ion operation.

Positive Ion

The positive ion reference file is called NAICS and contains the caesium ion mass at 132.9054. This peak may be produced in the positive ion spectrum by adding a very small quantity (~0.01 µg/µl) of caesium iodide to the solution, but can also complicate the spectrum by giving peaks containing combinations of Na and Cs in addition to the simple NaI peaks.
The negative ion reference file is called NAINEG.
Chromatographic Interfacing

Electrospray ionisation can be routinely interfaced to reversed phase and normal phase chromatographic separations. Depending on the LC pumping system, chromatography column and Quattro II set-up, there are some basic options:

- **Capillary chromatography separations** employing 1mm diameter (and smaller) columns can be interfaced directly to the electrospray probe. Typical flow rates for such columns may be in the region of 3-50 µl/min. It is suggested that a syringe pump is used to deliver these constant low flow rates through a capillary column. Alternatively, accurate pre-column splitting of higher flow rates from reciprocating pumps can be investigated.

  In all cases, efficient solvent mixing is necessary for gradient elution separations. This is of paramount importance with regard to low flow rates encountered with capillary columns. HPLC pump manufacturers’ recommendations should be heeded.

  The triaxial flow probe can be of value in running the capillary LC-electrospray systems. A make-up flow of organic solvent into the Quattro II triaxial-flow probe can reduce any sensitivity variations as a gradient elution programme proceeds and may also enhance the electrospray ionisation performance.

- **Microbore (2.1mm diameter) reversed phase columns** are gaining popularity for many separations previously addressed by 4.6mm columns. Typical flow rates are 200-400 µl/min. With a standard nebuliser assisted electrospray set-up it is recommended that a simple T-junction split should be arranged after the column so that 10-40 µl/min is transferred to the source with the remainder going to a fraction collector, a UV detector or to waste.

  A UV detector may be placed in-line to the Quattro II probe. However, ensure that the volume of the detector will not significantly reduce the chromatographic resolution. Whenever a UV detector is used, the analogue output may be input to MassLynx for chromatographic processing.

  The Megaflow option allows the direct interfacing of 2.1mm columns to the electrospray source.

- **The interfacing of 4.6mm columns to the electrospray source** can be achieved by either flow splitting or using the Megaflow electrospray facility.

  Conventional reverse phase and normal phase solvent systems are appropriate for LC-electrospray. However, involatile buffers should be avoided.

  Trifluoroacetic acid and triethylamine (TEA) may be used up to a level of 0.05%. If solvents of high aqueous content are to be used then tuning conditions should be appropriate for the solvent composition entering the source.
Higher source temperatures (100°C) are also recommended for high aqueous content solvents. THF should not be used with peek tubing.

**LC-MS Sensitivity Enhancement**

The sensitivity of an LC-MS analysis can be increased or optimised in a number of ways, by alterations to both the LC operation and the MS operation.

In the LC area some examples include the use of high resolution columns and columns with fully end capped packings. For target compound analysis, techniques such as trace enrichment, coupled column chromatography, or phase system switching can have enormous benefits.

Similarly, the mass spectrometer sensitivity can often be significantly increased, for instance by narrow mass scanning or by single ion recording techniques.

Careful choice of the solvent, and solvent additives or modifiers may also prove important.

**Running CE Samples**

It may be necessary to turn off both the source and nebulising gas during sample injection onto the column, and turned on again once a CE voltage has been established, to obtain reproducible sample injections.

CE capillary regeneration using molar NaOH should only be undertaken with the probe out of the source. However, it may be possible to carry out “minor” regenerations using plugs of 0.1M NaOH with the probe in the source without significant reduction in the time between source cleaning.
Atmospheric Pressure Chemical Ionisation

Introduction

Atmospheric Pressure Chemical Ionisation (APcI) is an easy to use LC-MS interface that produces singly-charged protonated or de-protonated molecular ions for a broad range of involatile and thermally labile analytes.

The ability to operate with 100% organic or 100% aqueous mobile phases at flow rates up to 2 ml/min makes APcI an ideal technique for standard analytical column (4.6mm i.d.) normal phase and reverse phase LC-MS.

The Quattro II APcI interface consists of a heated nebuliser probe and the standard atmospheric pressure source configured with a corona discharge pin. Mobile phase from the LC column enters the probe where it is pneumatically converted into an aerosol and rapidly heated into the vapour/gas phase at the probe tip. Hot gas from the probe enters the heated volume of the source which contains the corona discharge pin typically maintained at 3kV.
Mobile phase molecules rapidly react with ions from the corona discharge to produce stable reagent ions. Analyte molecules introduced into the mobile phase react with the reagent ions at atmospheric pressure and typically become protonated (in the positive ion mode) or deprotonated (in the negative ion mode). The sample and reagent ions pass through the counter electrode prior to being expanded through a sample cone and skimmer assembly into the mass spectrometer.

**Preparation**

**Converting the Source**

Refer to “Routine Procedures” and convert the instrument for APcI operation.

*The source can be converted from electrospray operation without venting the instrument.*

On the MassLynx tune page, select **Ion Mode** and choose **APcI+**.

Set **Source Temperature** to 150°C.

APcI operation may begin once the analyser pressure is in the $10^{-5}$ mbar range.

**Checking the Probe**

Check that the probe heater is off by setting **APcI Probe Temp** to 20°C.

Using the colour coding system, connect the gas supply lines to the APcI probe as shown on the first page of this chapter.

Remove the probe tip assembly by carefully pulling the metal shroud off the peek shaft.

Ensure that 0 to 0.5mm of fused silica is visible at the tip of the protruding stainless steel capillary.
Connect the LC pump to the probe with a flow of 50:50 acetonitrile:water at 1 ml/min.

Check that the liquid jet flows freely from the end of the capillary and the LC pump back pressure reads in the range 250-400 psi.

Check that the nitrogen supply pressure is 7 bar (100 psi).

Select **Gas** and turn on **ESI Gas 1**.

*The legend for this command may have been changed by previous users, for example to **Nitrogen**.*

Check that the liquid jet converts to a fine uniform aerosol.

Switch off the liquid flow.

Fully open the sheath gas regulator on the front panel and check that a sheath gas flow of 200 l/hour is obtainable. Reset the flow to its normal operating rate of 50 l/hour.

Select **Gas** and turn off **ESI Gas 1**.

Reconnect the probe tip assembly and insert the probe into the source.
**Checking the Source**

Using the colour coding system, connect the drying and exhaust gas supply lines to the source.

Select **Gas** and turn on **ESI Gas 1**.

Set the drying gas regulator on the front panel to read 400 l/hour.

Check, using soap solution, that there are no gas leaks.

Reduce the drying gas to 200 l/hour and set **Source Temperature** to 150°C. *The source should be able to reach this temperature and regulate at this value.*

Connect the high voltage BNC lead to the source and select [Operate].

With **Corona** and **HV Lens** set to zero, check that the **Cone** readback on the source page is reading the correct set value.

Set **HV Lens** to its maximum value and check that the readback reads approximately 2.00.

Set **HV Lens** to zero and **Corona** to 4.0 (its maximum value).

Check that the **Corona** readback is approximately 4.00 and the **HV Lens** readback is no greater than 0.30.
Checking the Probe Temperature Control and Trip Circuit

The APcI probe incorporates a temperature sensor that, once inserted, trips the power to the probe heater if either the sheath gas or the nebuliser gas fails, or is not connected during operation. To test the probe temperature control:

Select **Gas** and turn on **ESI Gas 1**. Set the source drying gas to 200 litres/hour and the sheath gas to 50 litres/hour.

Connect the probe cable and check that the **APcI Probe Temp** readback reads approximately 20°C.

Set **APcI Probe Temp** to 400°C and start the LC pump at a flow of 1 ml/min (50:50 acetonitrile:water).

*The APcI Probe Temp readback should stabilise and regulate at a value of approximately 400±10.*

Repeat this test at a probe temperature of 650°C.

To test the probe temperature trip circuit:

Set the probe temperature to 650°C with the nebuliser gas on, the sheath gas at 50 litres/hour, the drying gas at 200 litres/hour and an LC flow of 1 ml/min, as described above.

When the **APcI Probe Temp** readback stabilises at 650°C select **Gas** and turn off **ESI Gas 1**.

Without delay, turn the LC flow to zero and monitor the **APcI Probe Temp** readback.

Check that the probe temperature remains at 650°C (±10°C) for approximately 30-40 seconds, and then starts to fall to a value ≤400°C in 30 seconds.

**Caution:** If the probe temperature does not start to fall after 40 seconds, unplug the probe immediately and consult Micromass.

When the probe cools to approximately 200°C, select **Gas** and turn on **ESI Gas 1**.

Check that the probe temperature starts to rise after approximately 30 seconds.

Turn on the LC flow at 1 ml/min and ensure that the probe temperature regulates at 650°C.
Obtaining a Beam

Set **Source Temperature** to 150°C

Insert the probe into the source.

Set **APcI Probe Temp** to 20°C with no liquid flow and **ESI Gas 1** off.

Set all the **Source** and **MS1** parameters as shown.

When the source reaches a temperature of 150°C:

Select **Gas** and turn on **ESI Gas 1**. Set the drying and APcI sheath gases to 200 litres/hour and 50 litres/hour, respectively.

Select one of the peak display boxes and set **Mass** to 50 and **Span** to 90.

Select **OPERATE**.

Increase **Gain** on the peak display box in the range 10-100 until ion peaks become clearly visible.

Optimise **Corona** so that the peaks reach maximum intensity (typically 2.8 to 3.4kV in positive ion mode, 1 to 2kV in negative ion mode).

Set **APcI Probe Temp** to 500°C and start the LC pump at a flow of 1 ml/min.

*At this point the ion peaks may decrease in intensity.*

Reoptimise the intensity of the peaks using the off-axis mechanism.
Adjust the screws alternately, in diagonally opposing pairs, to move the inner source assembly. Typically the beam intensity optimises with the eccentricity shown, though the angular position of the offset is variable.

As a guide, 50:50 acetonitrile:water typically gives ions at masses 19, 42, 60 and 83, corresponding to H₂O⁺, CH₃CN⁺, CH₃CN.H₂O⁺ and (CH₃CN)₂H⁺. In order to see all of these ions it may be necessary to reduce APcl Probe Temp to below 200°C.

Finally, retune all the parameters described in this section to optimise the intensity of the background (reagent) ions.

**Caution:** Switch off the liquid flow and allow the probe to cool (<100°C) before switching off the nebuliser gas and removing the probe from the source.
Calibration

Having obtained a stable APcI beam, proceed as follows to calibrate the instrument in APcI mode:

Set **APcI Probe Temp** to 200°C.

Acquire in full scan centroid mode over the mass range 100-1200 Da in 3 seconds.

Set the flow rate to 1 ml/min and inject 10µl of an acetonitrile:water solution containing 300 pmol/µl of PEG 200, 600 and 1000.

1.2mg of PEG 200, 3.6mg of PEG 600 and 6mg of PEG 1000 dissolved in 20ml of 50% aqueous acetonitrile produces a calibration solution of concentration 300 pmol/µl of each component.

Using a spectrum from the top of the chromatogram peak, perform a dynamic calibration using the PEGH calibration file.

*It is important to note that this procedure occasionally gives rise to ammonia adducts on each peak. In this case the PEGNH calibration file should be used.*

Hints for Sample Analysis

**Tuning**

- Start by tuning on the solvent ions.
- It is generally found that the most significant analyte tuning parameter to adjust following tuning on the solvent ions are **Cone** and **Skimmer Lens Offset**.
- Fine tuning on the analyte of interest can be performed either by large loop injections (100µl) or by constant infusion in the mobile phase (typically at analyte concentrations of a few ng/µl).
- 10µl loop injections can be monitored using real time chromatogram updates.

**Mobile Phase**

- The choice of mobile phase is an important compound specific factor in APcI, for example steroids prefer methanol:water mixtures as opposed to acetonitrile:water.
- Analyte sensitivity is also dependent on mobile phase composition, which can be varied from 100% aqueous to 100% organic for any particular mixture.
**Probe Temperature**

This can be a critical factor for some analytes.

- **Involatile samples** (for example steroids) generally require high probe temperatures (>400°C).
- **Volatile samples** (for example pesticides) can be analysed with low probe temperatures (<400°C).
- In some cases, too high a probe temperature can lead to thermal degradation of labile samples.

**Drying Gas and Sheath Gas Flow**

Sample sensitivity is dependent on both of these parameters.

- **Involatile samples** may require a sheath gas flow (50-100 l/hour) to prevent chromatogram peak tailing.
- Although a drying gas flow of approximately 300 l/hour is adequate for most samples, the sensitivity for some analytes can be optimised with zero drying gas and a moderate or high sheath gas flow (100-200 l/hour).

**Sample Cone and Skimmer Lens**

These two parameters are of paramount importance for sample analysis since they critically influence the transmission (focusing) and fragmentation of analyte ions.

- In order to obtain maximum sensitivity, **Skimmer Lens Offset** must be set typically to 1-5 volts.

The value of **Cone** determines whether the resulting analyte mass spectra will be “soft” (a protonated molecular ion base peak) or fragmented (consisting of diagnostic fragment ions).

- To obtain soft ionisation, **Cone** should typically be set to 20.
- Most analytes will undergo significant fragmentation with a **Cone** value of 50.

**Skimmer and RF Lens**

These parameters need not be adjusted during day to day operation. Values of 1.5 and 0.2 respectively are typical and, once set, ought not to vary significantly.
**Source Voltages**

The following illustration shows the various components of Quattro II’s ion optical system. The name in the table’s first column is the name used throughout this manual to describe the component. The second column shows the term used in the current MassLynx release.

The voltages shown are typical for an instrument in good condition. The polarities given are those actually applied to the electrodes. Only positive values need be entered via the tune page.

<table>
<thead>
<tr>
<th>Component</th>
<th>Tune Page Name</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corona (kV)</td>
<td>Discharge Needle</td>
<td>+3.2</td>
<td>-1.5</td>
</tr>
<tr>
<td>HV Lens (kV)</td>
<td>Counter Electrode</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cone (V)</td>
<td>Sample Cone*</td>
<td>+25</td>
<td>-25</td>
</tr>
<tr>
<td>Skimmer Lens Offset (V)</td>
<td>Skimmer Lens</td>
<td>This lens optimises at a set voltage (typically 5V) higher than the sample cone. Skimmer Lens Offset is set from the Other menu on the tune page.</td>
<td></td>
</tr>
<tr>
<td>Skimmer</td>
<td>Skimmer</td>
<td>+1.5</td>
<td>-1.5</td>
</tr>
<tr>
<td>RF Lens</td>
<td>RF Lens</td>
<td>+0.2</td>
<td>-0.2</td>
</tr>
<tr>
<td>Not applicable</td>
<td>Differential Aperture</td>
<td>Ground</td>
<td></td>
</tr>
<tr>
<td>*The sample cone voltage is sample dependent. The values given are typical for low molecular weight singly charged ions. In-source fragmentation may be induced by increasing the sample cone voltage (typically 50V). The sample cone voltage may be ramped automatically with m/z to achieve optimum sensitivity across the m/z scale.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Removing the Probe**

After a session of APcI operation:

1. Turn off the LC flow.
2. Set **APcI Probe Temperature** to 20.
3. When the probe temperature falls below 100°C:
   - Turn off the gasses.
   - Remove the probe.
The SFC/APcI Interface

Introduction

The SFC/APcI interface can be easily configured for both capillary SFC (cSFC) and packed column SFC (pSFC).

The complete interface comprises:

- A dedicated SFC/APcI probe.
- A SFC reagent tee kit.

The SFC reagent tee, shown schematically overleaf, is used for both cSFC and pSFC. The branch of the tee comprises a stainless steel tube filled with HPLC grade water which generates a low level of water vapour into the drying gas at room temperature. The water vapour produces stable reagent ions in the APcI source (in a similar manner to the mobile phase in LC/APcI).

In this chapter, it is assumed that the reader is familiar with SFC technology and operation.
Disconnect the 4mm diameter drying gas inlet tube from the connector on the instrument’s front panel.

Place the SFC reagent tee directly in line with the drying gas tube as shown.

Attach the tee assembly to the front panel using the adhesive pad and cable tie provided.

Seal the APcl nebuliser gas lines on the probe and front panel using the 4mm plugs provided.

_Nebuliser gas is not required for SFC/APcl operation._
Capillary SFC

The SFC/APci probe, as supplied, is fitted with a SFC frit restrictor that provides a linear velocity of approximately 1.8 cm/sec for a 50µm i.d. column with CO₂ at 75 bar and 75°C.

*This restrictor is for cSFC use only.*

The capillary column should be connected to the free end of the restrictor using a ZDV union (not supplied) and graphite/vespel ferrules.

To operate in cSFC/APci mode:

Fill the reagent tee with HPLC grade water.

Set the drying gas to 200 l/hour and tune the source on the resulting reagent ions.

*It may take a few hours to displace any residuals, such as methanol or acetonitrile, left over from LC/APci.*

*A clean source will give reagent ions composed of proton hydrates at m/z values of 19, 37, 55 etc. depending on the value of the cone voltage.*

Set the APci sheath gas to 50 l/hour and the probe temperature to 300°C.

Pressurise the column with supercritical CO₂ and adjust the nitrogen gas flows and probe position to optimise the reagent ions.

“Loop injection” tuning on target analytes can be achieved by replacing the column with a 2 metre length of 50µm i.d. deactivated fused silica and injecting at a head pressure of 350 bar.

When replacing the frit restrictor, it is important to ensure that the frit is located in the probe tip heater as shown.
Packed Column SFC

Operation for pSFC is similar to cSFC, as described above. However, it is necessary to replace the frit restrictor with a high flow rate (typically 1-5 ml/min CO₂) integral restrictor and to adjust the position of the restrictor tip in the probe heater as shown.

Many chromatographers choose to make their own restrictors. To produce a restrictor for an isobaric SFC separation on a 4.6mm HPLC column at 2 ml/min CO₂ at 200 bar and 50°C, proceed as follows:

Take a 1.5 metre length of 75µm i.d. × 0.375mm o.d. deactivated fused silica capillary.

Carefully hold the capillary with a bunsen burner close to one end, and gently heat and draw the silica until two sections separate. Discard the short end length.

Carefully score the tapered end of the capillary approximately 10mm from the sealed tip using a ceramic fused silica cutter or equivalent. Remove the end piece by “snapping” at the score point.

Connect the resulting restrictor to the APcl probe as shown.

Connect the non-restricted end of the fused silica to the column outlet and set the APcl sheath gas to 50 l/hour and the probe temperature to 300°C.

Set the SFC pump to deliver 2 ml/min with an oven temperature of 50°C and observe the pressure readback as the system builds up to its equilibrium pressure.

If the pressure rises significantly above 200 bar:

Stop the CO₂ flow, cool down the probe and remove the restrictor.

Remove another 5mm to 10mm from the end of the restrictor using the ceramic capillary cutter.

Repeat the above procedure until an equilibrium pressure of 200 bar is achieved at a flow rate of 2 ml/min.
Electron Impact

Introduction

Sample molecules are admitted to the electron impact source by the GC interface, the particle beam interface, the solids insertion probe or the reference inlet system. The source is heated to ensure sample vaporisation, and the resulting gas phase molecules are ionised in collisions with high energy electrons released from the white hot filament. Ions are extracted from the source and into the analyser by the ion repeller and the focusing lenses.
Admitting Reference Compound

To admit reference compound to the EI/CI source for tuning and calibration purposes, or to admit volatile liquid samples, proceed as follows:

On the tune page select **Window** and **Pressures** to display the vacuum levels.

Make a note of the **Inlets** reading.

With the instrument in **OPERATE** select **Gas** and **Pump Out Reference Gas** on the tune window.

*This evacuates the sample reservoir and removes any previous sample.*

When the **Inlets** reading returns to its normal level:

Deselect **Pump Out Reference Gas**.

Inject about 5 microlitres of reference compound into the reservoir through the septum.

*To avoid long term contamination problems, only volatile compounds such as FC43 (heptacosa) should be injected.*

Select **Gas** followed by **Reference**.

To halt the flow of reference compound:

Select **Gas** and deselect **Reference**.

Tuning

Admit a suitable reference compound (normally FC43, heptacosa) as described above.

Set up a four box peak display on the tune page as shown.

Adjust the tuning parameters in the **Source** and **MS1** menus to optimise peak shape and intensity at unit mass resolution.

Note that the **Aperture** setting is inversely proportional to the size of the ion exit orifice. A setting of 0.0 is appropriate for EI operation.

The resulting peak display should resemble that shown.
Chemical Ionisation and Desorption CI

Introduction

Chemical ionisation is a "soft" ionisation technique in which sample molecules are ionised in reactions with ionised gas molecules. Ammonia, isobutane and methane are commonly used as reagent gases from which the reagent ions are generated. Ionisation of the reagent gas molecules is by electron bombardment within the ion chamber of the combined EI/CI source.

In order to achieve the relatively high pressures required within the ion chamber while maintaining an adequate vacuum elsewhere, the chamber must be made partially gas-tight. This is achieved by inserting a probe to close the probe orifice, and by reducing the diameter of the ion exit aperture. (A factory-fitted stainless steel ball permanently seals the probe orifice on machines not equipped with a probe.)

As sufficient electrons are unlikely to reach the electron trap under CI conditions, filament current is regulated using the total emission current and not the trap current as in EI operation.

Samples can be introduced using the solids insertion probe, the gas chromatograph, the reference inlet system or the particle beam interface.

For thermally labile samples, the desorption chemical ionisation option (DCI) is favoured. Samples are loaded onto a special probe and thermally desorbed into a chemical ionisation environment.
Connecting the Reagent Gas

When a new reagent gas cylinder is connected to the CI gas inlet at the rear of the instrument, it is essential to pump the gas line to the cylinder for a few minutes before the cylinder valve is opened.

**Caution:** take care, particularly when using ammonia as the CI reagent gas, that the oil in the backing pumps is not wet from previous LC operation. Rapid deterioration of the rotary pumps will occur under these conditions. Refer to "Routine Maintenance" for details of the gas ballast procedure.

On the tune page, select **Ion Mode** followed by **CI+** or **CI-**.

**Select Gas** and **Pump Out CI Gas**.

This process removes air from the line and checks for leaks which can have a very significant effect on CI performance.

Allow the gas line to pump-down and the inlet pirani to indicate a pressure of better than $10^{-2}$ mbar.

The regulator valve will normally be partially open at its correct operating setting. If the valve is to be opened fully in order to pump down the gas line more quickly, take a note of the amount of adjustment so that the original setting can be restored.

Check for leaks if acceptable pressure is not achieved after approximately 5 minutes.

Once the line has been pumped:

Deselect **Pump Out CI Gas**.

Set the cylinder pressure regulator to approximately 0.35 bar (5 lbf/in$^2$).

**Tuning**

With the EI/CI source fitted and a cylinder of reagent gas connected, proceed as follows:

Select **Ion Mode** followed by **CI+**.

*The CI reagent gas supply is automatically turned on, and the filament current is regulated by total emission rather than by trap current.*

**Aperture** (on the Source tune page) should be optimised using the smallest orifice (~10.0) though in negative ion operation some samples ionise more efficiently with the intermediate aperture (~5.0) selected.
If Save On Exit (on the tune page File menu) is enabled, previously selected values will be restored automatically whenever the mode is changed. The smaller ion exit aperture is also selected automatically.

It is essential, when using the standard EI/CI combined source, that the solids insertion probe (or, for DCI, the DCI probe) is fully inserted to ensure that the ionisation chamber is as gas-tight as possible.

CI gas flow to the source is increased by turning the regulating valve control knob anti-clockwise.

To reduce the pressure turn the knob clockwise and then select Gas and Pump Out CI Gas. After a few seconds deselect Pump Out CI Gas.

The source housing pressure is indicated both on the tune page and on the display. The indicated source pressure should not exceed $10^{-4}$ mbar.

*As the ion source is partially gas-tight, the actual pressure within the ion chamber is many times greater than that indicated on the read-outs.*

**Positive Ion**

Tune to one of the reactant gas peaks and tune the source and analyser parameters for optimum sensitivity.

*The ion repeller voltage should normally be set to zero.*

If isobutane is used as the reagent gas, then aim for a ratio of at least 2:1 between the intensities of masses 57 and 43.

For methane aim for a 1:1 ratio between masses 29 and 17.

Final tuning is best performed on sample peaks rather than reactant gas ions.

Octacosane ($C_{28}H_{58}$) gives a strong peak at $m/z$ 393 when admitted using the solids probe. For tuning at higher mass, FC43 (heptacosa) admitted via the reference inlet system (see “Electron Impact”) has a peak at $m/z$ 652

**Negative Ion**

*The ion repeller voltage should normally be set to a few volts negative as a starting point.*

Using FC43 (heptacosa) admitted via the reference inlet system (see “Electron Impact”) optimise the tuning voltages and the reagent gas pressure while monitoring masses 452 and 633.
Desorption Chemical Ionisation

To operate in the desorption chemical ionisation mode a DCI probe, complete with removable probe tip, is required in addition to the standard EI/CI source:

Tuning

Before commencing DCI operation the system should be tuned for positive ion or negative ion CI operation, as described in the preceding pages of this chapter.

Loading the Sample

The probe tip is equipped with a platinum coil onto which the sample is deposited. Before loading the sample, ensure that the coil has been flamed or cleaned with a suitable solvent to remove traces of previous samples.

*The DCI probe tip is fragile and should be handled with care.*
There are two recommended techniques for loading the dissolved sample onto the coil:

- The coil is dipped into the sample solution.
- A syringe is used to deposit a drop within the coil.

*Surface tension ensures that the space within the coil is filled with sample solution.*

*In either case it is wise gently to evaporate the solvent before inserting the probe.*

**Desorbing the Sample**

The sample is desorbed into the ion source when a heating current is passed through the coil. The DCI inlet editor allows this heating current to be programmed in up to five ramps.

The rate at which the coil is heated can be critical and varies with the type of sample. Some experimentation may be required to establish the optimum conditions for the desorption of individual samples. Turning **TIC Control** on allows the heating current to be regulated by feedback of the total ion current. See the MassLynx NT User's Guide for details.
Thermospray and Plasmaspray

Introduction

The thermospray ion source is essentially a very simple device. A heated metal capillary vaporises the HPLC eluent. The resulting aerosol spray passes from the vaporiser into a heated ion source. In this region desolvation occurs, sample ions are formed and are subsequently sampled through a small orifice into the mass analyser. The bulk of the vapour produced in the system is pumped from the source volume by a high capacity rotary pump.

Two methods of ionising a thermospray aerosol are available: classical thermospray (TSP) and discharge assisted thermospray. Plasmaspray (PSP) is a trade name for the generic technique of discharge assisted thermospray.
Thermospray

In classical thermospray, a volatile electrolyte (or buffer) is dissolved in the HPLC eluent. This electrolyte provides the primary source of reagent ions to ionise sample molecules. “Electrolyte ionisation” is only feasible with reverse phase chromatography where solvents of sufficient polarity to dissolve the electrolyte are employed.

Many factors influence fragmentation and sensitivity in thermospray ionisation:

- Concentration of the thermospray electrolyte.
- Volatility of the thermospray electrolyte.
- pH of the electrolyte solution.
- Temperature of the thermospray source and capillary.
- Strength of the retarding electrostatic field (repeller voltage).
- Distance between the vaporiser and the sampling orifice.
- Ion source pressure.

Plasmaspray

Plasmaspray ionisation is suited to normal phase HPLC as it does not require an electrolyte in the mobile phase. Plasmaspray is a gas phase ionisation technique similar in concept to chemical ionisation.

An electrical discharge from the discharge electrode causes ionisation of the solvent vapour, forming CI reagent gas ions which in turn ionise the sample. Positive ions are generally formed by protonation, negative ions by proton abstraction. The ionisation efficiency is governed by the relative proton affinities of the solvent vapour and the analyte.

Plasmaspray ionisation is “harder” than thermospray - it produces greater fragmentation giving more structural information. Sensitivity is optimised at 95% to 100% solvent vaporisation, significantly higher than for thermospray. Vaporiser temperatures for plasmaspray are therefore generally higher than those for thermospray.
Optimising Solvent Delivery Systems

Detection limits can be lowered by improving the signal to noise observed in total ion current and mass chromatogram traces.

Noise in thermospray chromatograms is attributable to two principle sources:

- Thermospray chromatograms exhibit high frequency noise with a period of a few seconds, attributable to instability in the vaporisation process occurring during thermospray ionisation. Fortunately, as the period of this noise is of a few seconds or less, significantly different from the typical peak width of an HPLC chromatographic peak, it is easy to eliminate this noise either with dynamic digital filtering or by post acquisition processing (smoothing).

- The second source of noise is attributable to instability in the rate of solvent delivery from the HPLC pump. The period of HPLC pump noise is typically somewhere between 10 and 20 seconds, very similar to the peak width of an HPLC chromatographic peak. In instances where HPLC pump noise is severe it can easily obscure small chromatographic peaks. By eliminating instabilities in the rate of solvent delivery it is possible to make marked improvements in detection limits.

High performance liquid chromatography pumps are designed to work at a back pressure of several thousand psi, a typical analytical HPLC column will generate a back pressure of this order. In so called “off-line” LC-MS, the HPLC pump is connected directly to the thermospray interface via a valve loop injector. In this instance the only back pressure against which the pump has to work is generated by the thermospray interface and in some cases the back pressure can be as low as a couple of hundred psi. Under these conditions any deficiencies in the HPLC pumping system will be accentuated. Consequently the signal to noise observed from loop injections is often worse than when the sample is injected on-column.

One method of overcoming this problem is to include a restrictor in the HPLC pumping system that is used for thermospray. The restrictor (typically a length of fused silica capillary) generates sufficient back pressure for the pump system to operate efficiently and smoothly even in the absence of an HPLC column. Multisolvent delivery systems for HPLC- MS modified with restrictor devices to enable smooth operation are now commercially available.
It is relatively easy to modify an existing HPLC pump by adding a simple home-made restrictor. The restrictor consists of a length of fused silica capillary of a diameter and length chosen to produce a back pressure of around 1500-2000 psi. This capillary is sleeved inside a piece of narrow bore PTFE tubing having an external diameter of 1/16". The restrictor assembly is inserted into the solvent delivery line before the valve loop injector using standard chromatography fittings (1/16" unions with metal nuts and ferrules). When the nut is tightened it compresses the ferrule onto the PTFE tubing which in turn compresses to grip the fused silica capillary.

### Post Column Addition of Thermospray Electrolyte (Buffer)

In thermospray ionisation it is necessary to have a volatile buffer dissolved in the fluid entering the interface. However, the buffers used in thermospray (ammonium acetate or ammonium formate) can sometimes degrade the performance of a HPLC column or of a chromatographic procedure. Also, the pH at which chromatography is optimised may not be the optimum for thermospray.

**Advantages**

- Permits independent optimisation of both HPLC eluent and TSP fluid conditions.
- Maximises the aqueous content of the TSP fluid in instances where this is desirable.
- Minimises the change in TSP fluid composition in gradient elution applications.
- Dilutes sample concentration before vaporisation thus reducing the probability of blocking or “wall coating” the interface capillary.
Disadvantages

- Band broadening effects.
- Sample dilution adversely affects the sensitivity of volume sensitive detectors such as UV.
- Auxiliary HPLC pump required.

When adding buffer or modifier post column it is important to minimise the dead volume associated with the mixing arrangement. Post column mixing tees are available commercially (Valco). HPLC columns terminated with combined end fittings and mixing tees are recommended. These are known as TCEF (Valco) column end fittings. It is also possible to obtain prepacked HPLC columns with TCEF end fittings from Phase Separations (UK) and from Hewlett Packard (USA).

HPLC Stationary Phases

To facilitate classical thermospray ionisation, the mobile phases that are used for LC-MS must contain an electrolyte or buffer, typically ammonium acetate or ammonium formate. Alternatively acidic or basic eluents can be employed.

In instances where post column addition is not used, the buffer solution will pass over the stationary phase of the HPLC column. The performance of uncapped stationary phases used in reverse phase chromatography can be degraded by buffer salts and ion pairing reagents that may be present in the thermospray mobile phase.

Moreover, silica based packing materials may be attacked both by basic and strongly acidic mobile phases causing the bonded phase to be stripped.

The use of such unstable materials for thermospray LC-MS may lead to premature blocking of the vaporiser. It is advisable, therefore, to use more stable stationary phases for LC-MS applications.

Silica based reversed phases that are fully end capped are recommended for general use. Where mobile phases of very high or low pH are to be employed, polymeric reverse phases are recommended (for example Hamilton PRP-1).
Simultaneous TSP/PSP and UV Detection

The response factor for a given compound in thermospray (TIC) can be significantly different from its UV absorbence at, say, 254nm. As a consequence the UV absorbence chromatogram and the TIC chromatogram for the same HPLC separation can look radically different. To eliminate any confusion and to add further specificity to the LC-MS analysis it is advantageous to include a UV detector in series with the mass spectrometer and thereby obtain both a UV and a TIC chromatogram.

This is easily achievable as UV absorbence detection is a non destructive analytical technique. MassLynx includes the facility to acquire analogue signals of the type generated by a UV detector and to acquire both UV and mass spectral data into the same datafile. In certain circumstances it is advantageous to use the mass spectral data for compound identification and the UV data for quantitation.

There are two ways of connecting the two detectors:

Series Coupling

The eluent from the column passes through the UV absorbence flow cell and then into the mass spectrometer. As all the sample passes through both detectors the sensitivity of each method is maximised.

However, if the UV flow cell is not tolerant of high pressures, it is likely to fracture. Under normal conditions thermospray interfaces generate back pressures from 500 psi to about 1500 psi, but this can be significantly exceeded should the capillary begin to block. Several manufacturers offer high pressure flow cells for their UV detectors (e.g. Waters 490 MS). A rating of 4000 psi is recommended.

Parallel (or Split) Coupling

The eluent from the column is divided, a portion going to the mass spectrometer and the residue to the UV detector. As the eluent is divided, the detection limit is reduced for both detectors. However, as there is no flow restriction on the outlet side of the UV flow cell, there is no back pressure build up.
The Repeller Electrode

The repeller electrode in the thermospray ion source is used for two purposes:

- To increase the sampling efficiency of ions from the ion source into the mass spectrometer
- To promote controllable fragmentation within the ion source.

The “downstream” repeller electrode used on the Quattro II thermospray ion source can be operated at high voltages, providing a high degree of control and fragmentation efficiency.

As a general rule, as the repeller voltage is increased from 0 to 350 volts there is an improvement in the extraction of ions from the source, thus maximising sensitivity.

At the higher repeller voltages (150-350V) fragmentation is promoted by CID processes occurring within the high pressure region (10mbar) of the ion source. This provides a controllable “structural probe”.

Comparing thermospray spectra obtained on a single quadrupole instrument at both high and low repeller voltages with spectra obtained on a triple quadrupole instrument at low repeller voltage, but at high collision energy, it is possible to determine that many of the fragment ions formed in a thermospray source at high repeller voltage are true daughter ions formed by CID processes.

It is possible to determine which ions formed in the ion source are in fact true daughters of the parent. Thus, by promoting the first “generation” of fragmentation products in the source and performing a second fragmentation in the collision cell it is possible to look, not only at daughter ions, but also at grand-daughter ions in a combined TSP LC-MS-MS experiment.

Gradient Elution HPLC-MS

When performing gradient elution thermospray or plasmaspray LC-MS the solvent composition of the liquid entering the thermospray vaporiser changes with time. In reverse phase chromatography this is typically from a predominantly aqueous solution to a predominantly organic solution. As the composition of the mobile phase changes so does its latent heat of vaporisation.

To achieve smooth and stable ionisation it is therefore necessary to adjust the temperature of the vaporiser with change in solvent composition. In a typical gradient elution reverse phase LC-MS run the temperature of the vaporiser would decrease as the run progresses. Quattro II’s thermospray interface can easily be programmed to run temperature gradients.
Guidelines

Optimisation of Parameters

Before embarking on on-line LC-MS experiments it is generally advisable to perform loop injections for the purpose of optimising the vaporiser temperature, the source temperature and the repeller electrode voltage. Loop injections for optimisation should, where possible, be performed with pure standards. If these are not available mixtures can be injected.

Optimisation of temperature (see following chapter) and repeller voltage should be performed using the mobile phase that is intended for on-line isocratic LC-MS.

If gradient elution LC-MS is envisaged then the optimisation process should be repeated using solvent compositions that are characteristic of the extreme ends of the proposed gradient. Using the two sets of optimised parameters it is then possible to program the vaporiser linearly between these two points. If a non linear gradient is proposed then more data points at different solvent compositions will be required.

Optimising Vaporiser Temperature

The thermospray ionisation process requires that the HPLC eluent be in a superheated, nebulised state upon leaving the vaporiser. If temperatures are lower than that required to produce a nebulised mist, liquid will spray into the source and sufficient desolvation of the droplets will not occur efficiently to promote gas phase ion formation. At temperatures higher than those required for nebulised mist formation a gas stream is formed where ion formation is unfavourable. In either case, ion formation depends on the extent of desolvation and the energy of the nebulised droplets.

Conversely in plasmaspray it is necessary to vaporise the liquid almost completely. The presence of droplets in the vapour stream passing through the source tend to destabilise the discharge.

Before injecting an analyte, the thermospray interface should be tuned by maximising the intensity of the solvent cluster ions. Quattro II should be scanned from approximately mass 20 to 150. While observing the solvent cluster ions, the vaporiser temperature should be varied to maximise the total ion current.

As the temperature increases so, generally, does the total ion current response until a maximum is reached after which the ion yield will begin to decline. Additionally, as the maximum of the TIC response curve is approached the ion current can become unstable.
To achieve a satisfactory compromise between TIC response and stability it is recommended that the vaporiser temperature be set about 5 degrees below the temperature of maximum TIC. When this condition has been achieved the system is ready to inject an analyte.

It should be remembered that the optimum temperature for solvent ion generation may not necessarily be the same as that for the sample, so a second stage of “fine tuning” may be required.

When starting to analyse a sample of unknown concentration or composition it is sensible to start the analysis at a relatively low vaporiser temperature. If no ions are observed then the temperature should be raised in small steps until the onset of thermospray ionisation. If the sample is suspected to be very concentrated it is a wise precaution to dilute it before injecting.

**Chromatography**

When performing on-line LC-MS, the importance of good chromatography cannot be ignored. Quattro II’s specificity allows coeluting peaks to be resolved using mass chromatograms. This, however, should not be an excuse for sloppy chromatography. Although coeluting peaks do not generally cause problems, broad peaks will decrease the sensitivity of the system. Chromatography ideally should be optimised to produce the “sharpest” peaks possible.

Short column or “flash chromatography” is recommended to minimise analysis time by exploiting the specificity of MS or the ultra specificity of MS-MS.

**Choice and Optimisation of Electrolyte**

To achieve the maximum sensitivity in classical thermospray analysis it may be necessary to add buffers and/or modifiers post-column to achieve the optimum conditions for thermospray analysis. For example, reducing the pH of the HPLC eluent after the chromatographic step increases the efficiency of positive ion formation for many polar analytes.

When analysing ionic compounds by thermospray LC-MS it should be remembered that the thermospray electrolyte can interact with the analyte to suppress ionisation. For example, if cationic samples are analysed, raising the pH will suppress the ion yield of the cation. Conversely, if anionic compounds are being analysed lowering the pH by the addition of acid will suppress the yield of the anion. The presence of a thermospray buffer such as ammonium acetate can also suppress the ionisation of both cationic and anionic analytes.

When optimising a thermospray analysis the concentration of the ionising buffer should also be considered. As a general rule, the more concentrated the buffer the more fragmentation will be produced. For ammonium acetate, a typical working range to evaluate would be from 0.02M to 0.2M.
**HPLC Solvent**

The choice of solvents is also important when optimising the sensitivity of classical thermospray ionisation as the HPLC solvent chosen must also readily dissolve the buffer. The two most common HPLC solutions which fit this criteria are methanol in water and acetonitrile in water, both of which are used in reverse phase chromatography.

It is generally found that methanol is a better solvent when positive ions are to be analysed and acetonitrile is a better solvent when negative ions are to be analysed. Moreover, ions of either polarity are formed in higher yield when the aqueous content of the solution is maximised.

It is possible to add both organic and aqueous solvents after the chromatographic step, by post column addition, to achieve the ideal “solvent blend” for the desired mode of thermospray analysis.

TSP ion formation is favoured in the presence of water. This is probably due to the fact that water is an excellent solvating agent for ammonium acetate and increasing water content enables the solution to have a larger conductance. The increased conductance at high percentages of water results from the increasing abundance of NH$_4^+$ ions and (negative) acetate ions in solution. The net result is an increase in analyte ions formed due to the increase in reagent ions in solution.

**Sample Filtering**

Dirty samples, particularly biological extracts injected at high concentration, can soon start to block the system.

Solutions that are to be injected into a thermospray interface should be filtered prior to injection. Alternatively an in-line HPLC filter should be included in the system between the valve loop injector and the thermospray interface.
Fast Atom Bombardment

Introduction

Fast atom bombardment (FAB), in its original form, is a technique in which sample molecules are ionised in collisions with fast moving neutral atoms. A variation on this technique is secondary ion mass spectrometry (SIMS), in which the bombarding (or primary) beam consists not of neutrals but of ions.

Although not strictly accurate, the term “fast atom bombardment” is still widely used to describe the general process. CsSIMS is a more precise term for the system used on Quattro II, in which caesium ions are used as the primary beam.

In the conventional FAB system, samples are deposited on the tip of a probe which is then admitted to the ion source via an insertion lock. In the dynamic-FAB mode, sample molecules are swept onto the tip of a hollow probe by a continuous flow of pumped solvent.

Where confusion between these two modes of operation might otherwise arise, conventional non-flow FAB is referred to in this manual as “static-FAB”
Hardware

Quattro II's fast atom bombardment system is a straightforward technique requiring the following hardware:

- A FAB ion source complete with insertion lock.
- A caesium gun.  
  
  This gun is permanently mounted onto the source region’s top cover.
- A static-FAB sample probe.

Additional hardware is required for dynamic-FAB operation:

- A dynamic-FAB sample probe.
- A sample introduction system (user supplied).
Dynamic-FAB

Overview

The dynamic-FAB interface has a number of clear advantages over static-FAB. These include:

- Higher sensitivity (10-fold to 200-fold better).
- Reduced suppression effects in mixture analysis.
- Improved quantitation.
- A wider range of applications, in particular LC-MS and capillary electrophoresis-MS interfacing.

The Probe

Sample is admitted to the ion source by the sintered wick probe. The sintered wick probe consists of a sintered stainless steel body, acting as a wick, with a stainless steel 8 micron mesh screen held tightly against a dome by the stainless steel end cap. The end cap also acts as a shield to prevent the primary ion beam hitting the sintered wick and producing "ghost" peaks.
Matrices and Solvents

In static-FAB analysis, the choice of matrix and solvent is critical as there is a strong compound-dependence. In contrast, in dynamic-FAB analysis the matrix is much less critical, probably because it is only present at 1-5% v/v.

The dynamic-FAB system works equally well with reverse phase and normal phase solvent systems and is suitable for both isocratic and gradient elution experiments. Low levels of modifiers such as acids, dilute buffers or salts, and compounds such as SDS or polyethylene glycols, can be included in the solvent systems without problem.

Flow Modes

The dynamic-FAB probe can be used in a wide range of applications. These can be classed into four operating modes:

- Continuous Introduction.
- Loop Injection.
- LC-MS.
- Capillary Electrophoresis-MS.

Continuous Sample Introduction

Continuous sample introduction can be employed when a steady signal is required, for instance in accurate mass analysis. This mode of operation is also useful in real time monitoring of chemical or biochemical reactions.

Loop Injection

Loop injection mode is useful when rapid sample throughput is required, or when quantitative studies are to be made. It is probably the method of choice if the sample is reasonably pure or consists of a simple mixture. Because of the excellent stability of dynamic-FAB, background subtractions can be carried out reliably and routinely to yield spectra of excellent quality. The LC pump should be set to deliver approximately 5 µl/min.
LC-MS

The dynamic-FAB probe can be used as an interface with liquid chromatography columns of almost any size, ranging from open tubular (10µm internal diameter) to analytical or larger.

At one extreme, the OTLC columns require a make-up flow. This situation is ideal for the post-column addition of matrix.

Packed fused silica columns of 320µm i.d. have an optimal flow rate of 5 µl/minute, which is ideal for direct coupling to the dynamic-FAB system.

Microbore, analytical and larger i.d. columns require a post-column split to give a suitable flow to the dynamic-FAB probe. Although this is inherently wasteful of sample, it has the advantages that standard LC hardware can be used, and many LC separations have been well characterised using these columns.

Capillary Electrophoresis

Capillary electrophoresis can be interfaced to the dynamic-FAB system. There are many similarities with OTLC-MS, since in both cases a make-up flow is required.

Two types of CE-dynamic-FAB interface have been described, based on a liquid-liquid junction and on a coaxial design. Negligible band-broadening has been observed with the coaxial interface. Data demonstrating 600,000 theoretical plates have been obtained, as below.

![CE-MS chromatogram of two peptides.](image)
Flow Splitters

When interfacing microbore or larger LC columns to the mass spectrometer using a dynamic-FAB probe, a suitable type of splitter device should be chosen. The four types of splitter routinely in use are:

- A tee plus a length of silica acting as a restrictor.
- A needle valve.
- A balance column system.
- A pneumatic splitter system.

The first two types of splitter are relatively simple, but can be difficult to control. The balance column system has the advantage that it can be used as a pre-column split in gradient elution experiments. The pneumatic splitter system has the advantage that it is easily controllable.

Matrix Introduction

For convenience it is best, if possible, to include the matrix (commonly glycerol) in the LC solvent reservoirs.

If this is unsatisfactory, then a separate matrix introduction system will be required. The matrix can be added post-column at high flow rates (microbore or analytical columns) or at low flow rates (capillary electrophoresis, OTLC or packed fused silica columns).

If low flow rate mixing is required, care must be taken to avoid band broadening.

Another approach could be described as “on target” mixing, in which dual silica capillaries or coaxial capillaries carry sample and matrix to the probe tip prior to mixing.

An easily-controllable solvent delivery system should be chosen for the matrix flow.
LC-MS Sensitivity Enhancement

The sensitivity of an LC-MS analysis can be increased or optimised in a number of ways, by alterations to both the LC operation and the MS operation.

In the LC area some examples include the use of high resolution columns and columns with fully endcapped packings. For target compound analysis, techniques such as trace enrichment, coupled column chromatography, or phase system switching can have enormous benefits.

Similarly, the mass spectrometer sensitivity can often be significantly increased, for instance by narrow mass scanning or by single ion recording techniques. Careful choice of the solvent, matrix and solvent additives may also prove important.

Accurate Mass LC-MS

Accurate mass measurement on LC-MS samples can most readily be performed using a suitable reference compound (typically a mixture of suitable polyethylene glycols or PEG monomethyl ethers) added post-column. The use of microbore or larger LC columns in conjunction with a post-column mixer and a pneumatic splitter provides a very easily controlled system for this purpose.

A system for accurate mass LC-MS
Installation

The caesium gun is mounted on the top cover plate, and need not be removed except when replacing the caesium pellet (see “Routine Maintenance”).

Referring to “Routine Procedures”:

Remove the installed source and, if necessary, the RF lens and adapter flange.

Check that the non-API guide rods and the differential aperture are fitted.

To fit the FAB source:

Remove the FAB source from its stand and install it into the housing.

Secure the FAB source with the two black screws.

Insert the aluminium tube at the end of the FAB pumping line into the pumping port on the bulkhead.

Ensure that the ball valve on the insertion lock is closed.

Connect the FAB source end flange to the sockets marked “EI/CI Source” on the instrument bulkhead.

Pump down the instrument as described in “Routine Procedures” and check that the source vacuum attains better than 10\(^{-5}\) mbar.

Operation

Inserting the Probe

Caution: Damage to the instrument may occur if the insertion lock is operated with the probe incorrectly positioned.

To insert the probe into the ion source:

Check that the lever on the probe guide is in the clockwise position.

Insert the probe into the insertion lock until the probe reaches the first stop.

Check that the pumping line is connected to the port on the Quattro II bulkhead.

On the tune page select **Pump Probe** on the **Gas** menu, or simply press the PUMP PROBE switch.

If required the vacuum levels can be displayed by selecting **Pressures** on the **Window** menu. **Inlets** shows the pressure in the insertion lock pumping line.

When the inlet pressure falls below 5 \(\times 10^{-3}\) mbar:
Slowly open the quarter-turn ball valve.

Rotate the probe guide lever anticlockwise.

Slowly push the probe fully in.

**Withdrawning the Probe**

To withdraw the probe after use:

Withdraw the probe to the first stop.

Close the quarter-turn ball valve.

On the tune page deselect **Pump Probe** on the **Gas** Menu, or press the PUMP PROBE switch on the front panel.

Rotate the probe guide lever fully clockwise and withdraw the probe from the lock.

*Caution:* Damage to the instrument may result if the probe is removed without first closing the ball valve.

**Loading a Sample (Static-FAB)**

Clean the FAB probe tip with a suitable solvent (water for CsI).

*With new probe tips, it is recommended that the surface of the target is roughened with emery paper to improve the sample distribution over the target.*

Coat the target with sample dissolved in a suitable matrix.

*A suitable liquid matrix for many peptides is glycerol or thioglycerol (monothioglycerol). A common method of preparing samples is to coat the probe tip with the liquid matrix, deposit a small amount of solid sample into the matrix and then stir the mixture using a glass rod.*

Reinsert the probe.
Tuning

With the FAB source fitted, select **Tune Mass Spectrometer...** on the Acquisition Control Panel and check that the FAB+ tune page is automatically loaded.

Deposit a sample of about 2µl of aqueous CsI onto the tip of the static-FAB probe.

Warm the tip to evaporate the water, leaving a layer of sample.

Insert the probe into the FAB insertion lock.

Select **Operate.**

**Caution:** To prevent rapid depletion of the caesium pellet do not set **Heater** above 2.5 amps

Allow a few minutes for the gun to stabilise at its operating temperature.

Optimise the intensity of the peak at m/z 133.

A small peak at m/z 133 will be observed without the probe inserted. This is due to primary ions from the caesium gun.

Tune the instrument across the desired mass range by examining peaks chosen from the CsI reference table (see “Calibration” below).
Calibration

Once the instrument has been tuned over the desired mass range a full scan acquisition should be made.

Compare the observed masses with those listed below.

If calibration is not satisfactory, recalibrate the instrument using the most suitable CsI reference table.

*MassLynx is supplied with several CsI reference tables to suit various mass ranges.*

<table>
<thead>
<tr>
<th>m/z</th>
<th>Intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>132.905</td>
<td>100.00</td>
</tr>
<tr>
<td>265.811</td>
<td>1.50</td>
</tr>
<tr>
<td>392.715</td>
<td>21.80</td>
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<tr>
<td>652.525</td>
<td>4.60</td>
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<tr>
<td>912.335</td>
<td>2.70</td>
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<tr>
<td>1172.145</td>
<td>1.28</td>
</tr>
<tr>
<td>1431.955</td>
<td>0.48</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>m/z</th>
<th>Intensity (%)</th>
</tr>
</thead>
<tbody>
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<td>0.69</td>
</tr>
<tr>
<td>1951.575</td>
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</tr>
<tr>
<td>2211.385</td>
<td>0.22</td>
</tr>
<tr>
<td>2471.194</td>
<td>0.25</td>
</tr>
<tr>
<td>2731.004</td>
<td>0.08</td>
</tr>
<tr>
<td>2990.814</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*Caesium Iodide Reference Peaks*

For the most precise applications, for example accurate mass dynamic-FAB LC-MS, the instrument can be calibrated using a mixture of caesium iodide and rubidium iodide. In this case use the CSRBl.REF reference table (see “Reference Information”).

Sample Acquisition

The instrument is now ready to perform sample analysis in either static-FAB or dynamic-FAB modes.

*The FAB source is equipped with a heater, though it is not normally necessary to heat the sample in static-FAB operation.*

Acquire data in the normal manner.
The Particle Beam Interface

Introduction

The LINC particle beam interface provides a straightforward way of interfacing liquid chromatography with quadrupole mass spectrometers. It can produce EI spectra of the eluting compounds that can be library-searched against standard libraries to aid sample identification. The system can also be operated in the CI mode.

The HPLC eluent enters the nebuliser which produces a well dispersed aerosol of very small droplets with a consequent large surface area to volume ratio, causing rapid solvent evaporation.

Samples, generally of lower volatility, remain as particles dispersed within the mixture of helium and solvent vapour.

A heater replaces the energy used as the solvent evaporates.

The mixture of particles and gas forms a supersonic jet sprayed out of the nozzle at the end of the desolvation chamber into a partial vacuum. The particles continue in a jet defined by the nozzle while the gas expands rapidly, allowing the particles to be separated from the gas by a skimmer cone pointed at the jet. Sample particles pass through a small aperture in the cone. This process is repeated at a second skimmer before the particles enter the ion source.
Installation

Hardware

The LINC particle beam interface consists of the following major modules:

- A desolvation chamber and momentum separator housing assembly.
- A nebuliser that connects to the HPLC system and fits into the end of the desolvation chamber.
- One “hot oil” rotary pump to pump away the (now gaseous) HPLC mobile phase. This pump can be also be used in conjunction with the thermospray / plasmaspray system.
- Additional vacuum pipework, with pirani gauge and controller, for second stage pumping.

Assembly

The interface is installed on the right hand side of the analyser housing.

Vent the system and remove the ion source as described in “Routine Maintenance”.

Remove the right hand flange from the vacuum housing by releasing the four socket-head screws.

Remove the blanking ball bearing from the port in the right hand source location yoke, by releasing the screw and removing the holding strip. Take care not to lose these three small components.

Insert the transfer tube, complete with spring and sliding sleeve, into the beam exit orifice on the particle beam interface. With the pumping tubes pointing downwards and with the interface slightly inclined to the horizontal (to prevent the transfer tube falling out) offer up the particle beam interface to the right hand port.

Ensure that the tube is correctly located before tightening the four screws to secure the interface.

If the system is to be operated in the CI mode, slide the sleeve towards the interface to cover the holes in the inner tube. For EI operation, ensure that these holes are clear.

Install the EI/CI source.

Reinstall the GC column, or blank off the interface.
Connect the momentum separator housing to the two rotary pump hand valves using the lengths of 38mm hose fitted to the separator. Ensure that the hot oil pump connects to the first stage.

If the pumps have not been left running, close both hand valves, connect the exhaust gas plumbing and start the two pumps. Operate these pumps in gas ballast mode for at least 30 minutes before operation of the interface.

Connect the desolvation chamber heater to the socket labelled “LINC” alongside the interface.

**Caution:** Care should be taken that the temperature selected on the tune page is appropriate for use with the LINC interface. A maximum of 50°C is recommended.

In the extreme right hand end of the desolvation chamber there is a hole into which the nebuliser fits. This should be fitted with a rubber bung. The instrument is now ready to be pumped down.

Open both rotary pump hand valves then select Other and Pump on the MassLynx tune window.

The nebuliser is fitted with a 1/8 inch pipe to connect it to a source of high purity helium at a pressure of at least 40 psi. The connection to the HPLC system is via a zero dead volume coupling which includes a removable 1/8 inch filter (5µm) to remove particulate matter. A spare filter is provided.

### Changing Between EI and CI Operation

In addition to the normal EI/CI changeover procedure:

Vent the system and remove the EI/CI source, as described in “Routine Procedures”.

If the system is to be operated in the CI mode, slide the sleeve towards the interface to cover the holes in the inner tube.

For EI operation, ensure that these holes are clear.

Re-install the EI/CI source.

### Removing the Particle Beam Interface

Removal of the particle beam interface is the reverse of the assembly procedure. Special care should be taken to ensure that a gas-tight seal is made when refitting the ball bearing to the port in the source location yoke.
Operation

Solvent Sparging

It is very important that the solvents used to form the mobile phase are thoroughly degassed. Sonication (even for long periods) in an ultrasonic bath is only effective for a short period of time afterwards. Continuous sparging with helium is recommended for best results. If solvents are not thoroughly degassed, air bubbles will form within the HPLC system producing variable pressure. Also, as the pressure drops dramatically when the mobile phase leaves the column just prior to entering the nebuliser, any dissolved air comes out of solution here.

Nebuliser

Adjustment

This all metal single grid nebuliser is optimised by adjusting the position of the \( \frac{1}{16} \) inch stainless steel capillary that delivers the mobile phase to the grid at the front end of the nebuliser.

Turn the 25 mm knurled adjuster fully clockwise (viewed from the rear).

Set the helium pressure to 2-3 bar (30-40 psi) and start the HPLC pump.

Slowly wind the adjuster anticlockwise so that the capillary moves away from the front grid and produces a uniform spray.

*The optimum position typically coincides with a sudden increase in the hissing noise from the nebuliser.*

The helium pressure and nebuliser position should be fine tuned for optimum sample sensitivity with the nebuliser operating in the particle beam interface.

Installation

With the interface and mass spectrometer pumped down remove the bung from the end of the desolvation chamber and replace it with the newly adjusted nebuliser.

*With a liquid flow rate of 0.5 ml/min the source pressure should stabilise between \( 10^{-3} \) and \( 10^{-4} \) mbar and the pressure at the second pump around 1 mbar.*

The nebuliser is fitted with its own O ring to produce a vacuum tight seal. Thus at any time it can simply be pulled in or out of the desolvation chamber.

It is recommended that the instrument is put into Standby mode whenever the vacuum to the desolvation chamber is broken. However it is not necessary to vent the instrument or interface.
It is recommended that the nebuliser is removed from the desolvation chamber at all times when the mobile phase and helium are not flowing. Otherwise the mobile phase remaining in the nebuliser and pipes will evaporate and be pumped away by the interface. This will lead to the deposition of any buffer salts used in the mobile phase within the HPLC equipment and also the drying out of the column. The nebuliser can be replaced by a rubber bung when the interface is not being used.

Desolvation Chamber Temperature

The purpose of the desolvation chamber heater is to replace the energy used by the evaporating droplets. Thus it is only necessary to set this to a few degrees above room temperature.

**Caution:** Take care to ensure that the temperature selected on the tune page is appropriate for use with the LINC interface. A maximum of 50°C is recommended.

Loop Injection

A quick and simple method to test the performance of the interface is to make direct loop injections without a column between the injector and the interface. Samples of known quantity enter the interface immediately allowing rapid assessment of performance.
Tuning

**EI**

On the MassLynx tune window, select **Gas** and **Reference** to turn on the reference gas.

Tune the instrument in the normal way, with solvent and helium flowing into the interface.

*The reference peaks will appear unstable (this is normal).*

*The repeller normally optimises between 5 and 10 volts.*

Note that there is one tuning optimum for both maximum peak size and maximum stability.

**CI**

Tune as for EI but use reactant gas peaks.

Operating Conditions

For normal operation, the following points should be checked:

- Reference gas is off (deselect **Gas** and **Reference** on the MassLynx tune window).
- The multiplier voltage is set to approximately 550V.
- The desolvation chamber temperature is set to the appropriate value, usually 30-40°C.

*This temperature is set using the Linc Heater parameter, located by selecting Heaters on the tune window.*

- The helium pressure is set to 3 bar (40 psi).
- The HPLC pump(s) are turned on and pumping smoothly.
- The nebuliser is optimised at the required flow rate.
- The source pressure is between $10^{-5}$ and $10^{-4}$ mbar.
- The pressure at the second rotary pump is about 1 mbar.
- The rotary pump gas ballast valves are open.

*This avoids condensation of solvent vapour.*

The mass range should, if possible, be chosen to exclude the components of the mobile phase to reduce the background. The scan time may be set to two or three seconds to smooth the noise (typically 300 amu/second).
**The TIC with no Sample**

The total ion current should settle to a constant, steady level when the mobile phase is flowing but no sample is entering the interface. Periodic variation is most likely due to the inability of the HPLC pump to deliver the mobile phase at a constant pressure. A gross periodic variation indicates that the pump should be serviced.

**Injection Technique**

Injection technique is important and those unfamiliar with the use of Rheodyne (and similar) valves should bear the following points in mind:

- Always use the correct syringe for injection. HPLC injectors require syringes with round tipped needles of a specific length. Using the wrong kind of syringe (especially a sharp pointed one designed to pierce the septum of a GC for example) can ruin a costly injection valve.

- The volume of the syringe should be two to three times bigger than the injector loop volume so that the loop is washed through by the sample when it is injected.

- Air bubbles must not be present in the injection.

- With the injector in the **LOAD** position, the loop should be filled twice with sample.

- Once the loop has been filled the injector should be switched to the **INJECT** position, left there for a few seconds, and then returned to the **LOAD** position, *without removing the syringe*.

- The needle should be wiped with a clean dry tissue between every operation.

- The syringe should be washed thoroughly with solvent whenever the sample is changed.

These points are equally valid for loop injections and injection via a column.
Assessment of Performance

With the following operating conditions the pressure at the second rotary pump should be about 1 mbar, and the source pressure about $3 \times 10^{-5}$ mbar.

- Mobile phase 1:1 methanol and water: 0.5 ml/min
- Helium pressure: 40 psi
- Desolvation chamber temperature: 40°C
- Source temperature: 250°C
- Photomultiplier voltage: 550 Volts

Using a sample of 10 ng/µl of caffeine dissolved in water and a 20ml injection loop, the injection should be clearly visible on the TIC as a well formed peak.

Injections of 1 ng/µl of caffeine, with all other conditions the same, should produce clear peaks on a mass chromatogram of 194.

In CI mode, a loop injection of 200ng of methyl stearate into a mobile phase of 0.5 ml/min. of hexane, and with isobutane as the reactant gas, should produce spectra with 299 the dominant peak.

Performance Discussion

In addition to the inherent variation of the strength of the signal that samples give in EI mode, there are additional sources of variation to be considered when the method of sample introduction is via particle beam.

The most important of these is the volatility of the solvent system used as the mobile phase. Basically the more volatile the mobile phase constituents are, then the better the particle beam system will work. Thus hexane has probably the highest transport efficiency for sample through the interface, and water the lowest.

The volume of water flowing into the desolvation chamber is the limiting factor on the ability of the interface to handle high flow rates. Thus high flow rates of normal phase (non-polar) solvents do not pose any problem, while reverse phase (polar) solvent systems, particularly systems including a high proportion of water, are subject to limitations.

The optimum flow rate for the LINC interface is therefore solvent system dependent, and no general figure can be provided. If the source pressure of the instrument rises to unacceptable values (say $10^{-4}$ mbar) then it is likely that the sensitivity is being compromised by the high flow rate of mobile phase into the interface.

There is also variation in the transport efficiency of the interface with sample volatility as quite volatile samples will obviously evaporate and be pumped away along with the solvent.
LC-MS Sensitivity Enhancement

The sensitivity of an LC-MS analysis can be increased or optimised in a number of ways, by alterations to both the LC operation and the MS operation.

In the LC area some examples include the use of high resolution columns and columns with fully endcapped packings. For target compound analysis, techniques such as trace enrichment, coupled column chromatography, or phase system switching can have enormous benefits.

Similarly, the mass spectrometer sensitivity can often be significantly increased, for instance by narrow mass scanning or by single ion recording techniques.

Careful choice of the solvent, and solvent additives or modifiers may also prove important.
The Gas Chromatograph

Introduction

The separative technique of gas chromatography (GC) is a process in which the compounds to be separated are partitioned between a mobile phase (the carrier gas) and the stationary phase (the liquid phase). It is the difference in partitioning between the stationary and the mobile phase that produces the separation.

The process takes place in the column which is essentially a tube through which a carrier gas flows. The stationary phase is present, in fused silica capillary columns, as a thin film coated on the inside of the wall and, in packed columns, as a coating on inert particles packed inside the column. The best results are generally obtained when using glass or fused silica as the tubing material.

Phases

There are many different stationary phases available each of which has its own effect on the separation of different classes of compounds. However, only a few phases are normally required for GC-MS use and should be chosen with separation efficiency and bleed level as main considerations. The most commonly used phases for GC-MS work are:

- **Non Polar**: OV 1, SE30, OV 101, DB5, OV 17, OV 1701
- **Medium Polar**: OV 225 & 275, Carbowax 20M, SP1000
- **Polar**: SP2330, Al₂O₃

Quattro II may be interfaced to one of several gas chromatograph systems which are controlled from the MassLynx data system. The MassLynx NT User’s Guide and the GC manufacturer’s documentation provide specific information concerning the set-up and operation of the installed system.
GC-MS Interfacing - Direct Coupling

The GC interface on Quattro II consists of a length of stainless steel tubing linking the oven and the mass spectrometer. The heated interface line passes through an oven fitted between the outer wall of the GC and the source housing.

Fused silica columns are passed through the tube and into the ion source. A Swagelock coupling fitted with a vespel or graphite ferrule provides a gas tight seal between the column and the tubing. **Do not use** stainless steel ferrules.

Other types of capillary column may be connected to a short length of fused silica capillary, using zero dead-volume connectors. The fused silica passes through the GC inlet into the ion source.
Column Installation

Fused silica columns are usually shipped with both ends sealed to protect the column.

Scratch the column with a glass knife, a couple of centimetres from each end, and snap off.

Slide a nut and a graphitised vespel ferrule over each end and snap off a further 2-3 centimetres of column.

This ensures that the column will be free of particles of graphite.

Examine the ends to ensure they are not splintered.

The inlet end should be installed first. Consult the GC instruction manual for the correct installation procedure.

Use a commercial leak checking solution such as “Snoop” to check for leaks at the injector.

Use a flow meter to set a gas flow of about 1 ml/min through the column.

The flow rate is determined by column head pressure and column length.

The septum purge flow should be about 1-1.5 ml/min.

If necessary, set any probe and source temperatures to a low value and allow to cool to below 100°C.

Turn off any gases flowing into the source.

Withdraw any probe and/or previously fitted GC column.

Vent the system as described in “Routine Maintenance”.

Remove the ion source.

With carrier gas flowing, feed the column through the interface and into the source region.
The length of the column required can be determined by observing the column end with the source removed. The column should be allowed to protrude beyond the interface until level with the outer rim of the ion exit aperture ring, as shown.

For CI operation, a more stable ion beam may be obtained by setting the end of the column closer to the centre of the source, as shown. The normal setting must be restored before resuming EI operation.

Make up the Swagelock coupling but do not tighten.

Mark the column with quick-drying paint (or typewriter correction fluid) where it enters the nut.

Take care to ensure that wet paint cannot be sucked into the transfer line when the system is pumped down.

Withdraw the column one or two centimetres.

Fit the EI/CI ion source.
Push the column into the interface until the white mark is in its original position.

Tighten the nut and ferrule to obtain a vacuum tight seal.

Pump down the instrument.

Due to the flow of carrier gas, ultimate pressures will, of course, be higher than when no column is connected.

### Injection Techniques

Good syringe technique is fundamental to obtaining reliable and reproducible results, especially in quantitative work. Poor injection techniques account for many of the problems encountered with capillary columns.

- Consistency is perhaps the most important factor in achieving reliable results.

- For 1 or 2 microlitre injections, a 5 or 10 microlitre syringe is best. For less than 1 microlitre, a 1 microlitre syringe should be used.

  *The plunger is withdrawn only a short distance and is unlikely to be damaged during the injection. It is quite easy to bend a fully withdrawn plunger when making the injection.*

- Keeping syringes clean and in good condition is also important.

  *Contamination due to sample accumulating in the syringe is a common cause of confusing results.*

The following injection procedure is not intended to be definitive, but should form the basis for the development of a consistent technique.

Before and after an injection is made, the syringe should be rinsed as follows:

Pour a few millilitres of solvent into a beaker.

Draw up into the barrel 4 or 5 microlitres of solvent and express it out onto a tissue.

*Care should be taken when using flammable solvents.*

Repeat this operation several times.

Pump the syringe with the needle remaining in the solvent.

Draw up a few microlitres and express onto a tissue.

Wipe the needle with the tissue taking care not to draw any solvent out of the needle by capillary action.

*The barrel of the syringe is now wet and the needle is full with pure solvent.*
To load the syringe:

Draw up into the syringe about 0.25 microlitres of air.

Draw up excess sample.

Position the plunger for the required amount of sample to be injected.

Always examine the quantity by viewing the syringe at eye level. Parallax can be the cause of misleading measurements.

Remove the syringe needle from the sample, wipe the needle with a tissue and draw up the plunger until the slug of sample can be seen inside the barrel.

This action will help prevent the loss of sample through evaporation.

Smoothly inject the needle through the septum whilst keeping a finger on the plunger to prevent blow-back.

Once fully inserted depress the plunger firmly and quickly.

Leave the needle in the injector for a few seconds. Usually five seconds is enough.

If the needle is withdrawn straight after injection, then the increase in the pressure in the injector, due to the evaporating solvent, may cause the sample to blow out through the hole in the septum.

Sometimes slow injections are required to concentrate the sample slug at the end of the column. Conversely, when thermally labile samples are being handled a faster injection is required to prevent thermal degradation in the injector. Your technique should be modified accordingly.

Rinse the syringe as described above.

Cleaning is especially important when changing samples, to avoid cross contamination. Build-up of organic material can be removed by washing the syringe and plunger in an ultrasonic bath. Alternatively, draw through the syringe a dilute chromic acid solution followed by a thorough rinse with water and then methanol (or other water-miscible solvent). Most syringe suppliers will advise on cleaning, and many offer a syringe reconditioning service.

Sometimes small fragments of septa lodge inside the needle and may block the bore. Silicone rubber may absorb some of the sample causing loss of sensitivity and cross contamination between samples. Needles can be cleaned out with fine wire.
Split Injection

The sample capacity of capillary columns is approximately 200ng per component. When operating in the split mode, only a small portion of the sample injected actually enters the column thus preventing column overloading. The remaining sample is carried out by the carrier gas through the split vent.

The ratio of inlet flow to column flow, commonly referred to as the split ratio, ranges from 10:1 to 500:1 depending on the column being used and the sample being analysed. A split of 25:1 is common for fused silica columns. Consult the GC instruction manual for the procedure to set up split ratios.

When operating in the split mode ensure that the correct liner is fitted in the injector.

Although the technique of split injection is fairly easy to master it does have two major disadvantages:

- During trace analysis the total amount of sample is severely limited. The analyst cannot afford to waste most of the sample through the vent.

- Split injection is a flash vaporisation technique and there is a possibility of sample discrimination when injecting wide boiling range mixtures.

To overcome these disadvantages, splitless injection is used.
Splitless Injection

The splitless injection technique allows virtually all of the sample to be concentrated at the head of the column, behind the condensed solvent band. In view of the beneficial solvent effect and much improved discrimination of wide boiling range mixtures, this technique is widely used in trace analysis work.

Concentration of the sample at the head of the column is achieved by stopping the gas flow through the split vent. None of the sample is vented away. After a short interval the vent flow is restarted.

In this mode larger quantities of solvent are used to provide the solvent effect. Also, component levels should be less than 50ng to avoid overloading the column.

When operating in the splitless mode:

Ensure that the correct injector liner is fitted.

Cool the oven to the recommended temperature for the solvent being used (see below).

Refer to the section of the MassLynx User’s Guide relevant to the installed GC and set the valve timings such that the split valve is closed at injection and opens one minute later.

Inject the sample. Large quantities should be injected over a period of 5-10 seconds.

Start the run at the required temperature.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Recommended Initial Oven Temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>10-30°C</td>
</tr>
<tr>
<td>Chloroform</td>
<td>25-50°C</td>
</tr>
<tr>
<td>Carbon Disulphide</td>
<td>10-35°C</td>
</tr>
<tr>
<td>Diethyl Ether</td>
<td>10-25°C</td>
</tr>
<tr>
<td>Pentane</td>
<td>10-25°C</td>
</tr>
<tr>
<td>Hexane</td>
<td>40-60°C</td>
</tr>
<tr>
<td>Iso-octane</td>
<td>70-90°C</td>
</tr>
</tbody>
</table>
Attention to fine detail is essential to obtain reproducible results and the following points should be noted:

- The inside of the injector must be kept clean.

- The capillary column inlet must be positioned above the split exit point. Refer to the GC manufacturers manual.

- The injection port temperature must be sufficient for efficient vaporisation of the sample, 200-300°C is usually adequate.

- Solvent quantities of 1-3 microlitres are required to obtain the solvent effect.

- Initial temperature of the column should be 10-20°C below the boiling point of the solvent to obtain the solvent effect.

- The injector should be purged through the split line 60-100 seconds after injection to prevent the solvent tailing.

**On-Column Injection**

The on-column injection technique has benefits when quantitative analysis is required, or thermally labile samples are to be analysed.

In on-column injections, the sample is injected in a liquid form straight into the column. Like splitless injection, on-column injections depend upon a solvent effect to concentrate the sample at the head of the column.

The sample is injected into the on-column injection port using a fine microneedle onto the head of the column, this being cooled by a stream of air to prevent vaporisation occurring in the injector.

Problems exist with this technique especially if dirty samples are used which will quickly affect the stationary phase. Also, only a limited number of stationary phases are suitable for repeated on column injections.
The Jet separator

The jet separator is mounted within the gas chromatograph with the column eluent fed directly to the separator inlet. The separator outlet is connected to a piece of fused silica capillary, sufficiently long to be fed through the transfer line and into the ion source in the manner described for capillary columns.

Caution: The SGE jet separator supplied with Quattro II is fitted with a 110 volt cartridge heater. The power for this heater should be obtained from one of the supplies for the GC’s detectors or auxiliaries.
When using the jet separator with packed GC columns, maximum enrichment and sample yield can be achieved by the careful selection of jet spacing. The gap between the jets often depends on the type of sample being analysed. A certain amount of experimentation may be required to find the optimum setting for a given set of chromatographic parameters.

For most purposes a gap of 0.3mm (0.012”) and a total flow rate of 15 mls/min will give satisfactory results. For information on setting the gap, refer to the manufacturer’s literature supplied.

Columns

Packed columns for GC-MS are generally 0.25" o.d. with an i.d. of either 2mm or 3mm. A 2mm diameter column will give slightly better separation with a lower flow rate and is thus preferred.

Column lengths may vary from 0.5 to 3 metres, and the shortest length that gives the required separation should be used. The selectivity of the phase should also be taken into account when deciding the length and diameter of the column.

Packing materials

The most commonly used supports are those made from diatomaceous earths. These come with different physical and chemical characteristics (for example, the surface area of the particle and the pH of the material).

Good packing materials for normal use are the Chromasorb W range that have been base treated, acid washed and treated with a silylating agent such as BSTFA or Dichlorodimethyl disilane. More specialised materials include the porous polymers (such as Porapak, Chromasorb 100 series and Tenax GC), molecular sieves (pore size range 4 - 13Å) and alumina. Porous polymers and alumina are useful for the separation of permanent gases.

Phases

In general, the phases used for capillary columns may also be used with packed columns.
The Solids Insertion Probe

Introduction

Solid samples, or liquid samples not sufficiently volatile for the reference inlet, may be admitted to the ion source using the solids insertion probe. The sample is loaded into a quartz sample cup which locates in the end of the probe. A thin strip of tungsten foil is used to hold the quartz sample cup firmly in position.

A heating element and thermocouple assembly is located at the end of the probe close to the sample cup.

A fine stainless steel capillary is wound close to the heater assembly to carry cooling water. If the probe is to be heated then a flow of cooling water must be maintained throughout the heating cycle to maintain fine control over the temperature at the tip as well as to enable rapid cooling of the probe. Failure to do so may result in the probe heater being damaged.

The probe may be heated rapidly by passing a current through the heater. The thermocouple monitors the temperature, and the heating current is controlled electronically to maintain the required program rate and final temperature.
Sample Loading

Two types of sample cup are available:

- For involatile samples, the shallow cup (type D) is recommended. Because the sample is close to the heated ion source it is often unnecessary to heat the probe.

- A deep cup (type C) is used for more volatile samples. The sample is deposited at the bottom of the cup so that it is away from the heating effect of the ion source. The sample can then be evaporated into the ion source using, if necessary, the probe heater.

Both types of sample cup should be pushed firmly into the probe tip so as to give good thermal contact with the heater element.

The sample cups can be loaded before or after inserting them into the probe.

Solid samples can be loaded into the cups using a thin piece of wire, or a drawn-out length of glass rod, to transport the sample into the cup.
Samples in solution are best loaded using a microsyringe.

Make sure that the sample is deposited at the bottom of the cup and not around the neck, taking care not to leave any air pockets.

If a number of samples is to be run sequentially it may be preferable to load all the cups and stand them in a holder that can be made for this purpose by drilling a series of 10mm deep × 2mm diameter holes in a block of metal.

Any solvent used in loading can be evaporated by placing the sample tube(s) in a warm place, for example on top of the GC oven. Care should be taken not to heat the holder too quickly so as to avoid blowing the sample out of the cup with solvent vapour.

Used sample cups should be cleaned in a hot flame and/or washed in solvent before re-use.
Water and Electrical Connections

If the probe is in constant use then its electrical and water supplies should be left connected. However, it is recommended that the water is disconnected overnight to minimise the risk of burst pipes through a rise in the water pressure. Disconnect the electrical connections so as to avoid heating the probe while the cooling water is disconnected.

To disconnect the water supply push back the collars on the water supply outlets, mounted at the rear of the bench, allowing the probe connectors to be withdrawn. Connection is simply the reverse: push back the collars, insert the connectors and release the collars.

Inserting the Probe

Caution: Damage to the instrument may occur if the insertion lock is operated with the probe incorrectly positioned.

To insert the loaded probe into the ion source:

Check that the lever on the probe guide is in the clockwise position.

Insert the probe into the introduction lock until the probe reaches the first stop.

Check that the water and electrical connections are made.

Check that the pumping line is connected to the port on Quattro II’s bulkhead.

On the MassLynx tune page select Pump Probe on the Gas menu, or simply press the Pump Probe switch.

The vacuum levels can be displayed by selecting Pressures on the Window menu. Inlets shows the pressure in the vacuum lock pumping line.

When the inlet pressure falls below $5 \times 10^{-2} \text{ mbar}$:

Slowly open the quarter-turn ball valve.

Rotate the probe guide lever anticlockwise.

Slowly push the probe fully in.
Heating the Probe

Source temperature is often sufficient to evaporate the sample, particularly if the shallower sample cup is used. If this is not the case, or if a more controlled evaporation is required, the probe inlet editor allows the temperature to be programmed in up to four ramps.

The rate at which the probe is heated can be critical and varies with the type of sample. Some experimentation may be required to establish the optimum conditions for the evaporation of individual samples. Selecting “TIC Control” allows the temperature to be regulated by feedback of the total ion current. The functions of all parameters are fully explained in the MassLynx NT User's Guide.

Caution: The standard probe should not be heated above 400°C. The optional high temperature probe may be heated to 750°C
Withdrawing the Probe

To withdraw the probe after use:

Allow the probe to cool to below 100°C.

*This protects the seals in the lock which might be damaged by high temperatures.*

Withdraw the probe to the first stop.

Close the quarter-turn ball valve.

On the MassLynx tune page deselect **Pump Probe** on the **Gas** Menu, or press the Pump Probe switch on the front panel.

Rotate the probe guide lever fully clockwise and withdraw the probe from the lock.

*Caution:* Damage to the instrument may occur if the probe is removed without first closing the ball valve.
Routine Maintenance

Introduction

Quattro II requires few maintenance procedures which must be carried out on a regular basis. These are listed at the end of this chapter under “Preventive Maintenance Check List”.

Other sections in this chapter relate to cleaning procedures and to the replacement of consumable parts, both of which may be required as a consequence of prolonged use. Information in the “Fault Finding” chapter may be helpful in identifying the procedures to follow.

Cleanliness and care are of the utmost importance whenever internal assemblies are removed from the instrument.

✔ Always prepare a clear clean area in which to work.

✔ Make sure that any tools or spare parts that may be required are close at hand.

✔ Obtain some small containers in which screws, washers, spacers etc. can be stored.

✔ Use tweezers and pliers whenever possible.

✖ Avoid touching sensitive parts with fingers.

✖ If nylon or cotton gloves are used take care not to leave fibres in sensitive areas.

✖ Do not use rubber gloves.

✔ Before reassembling and replacing dismantled components, inspect O rings and other vacuum seals for damage. Replace with new if in doubt.

**Warning:** Many of the procedures described in this chapter involve the removal of possibly toxic contaminating deposits using flammable or caustic agents. Personnel performing these operations should be aware of the inherent risks, and should take the necessary precautions.
Venting

The Quattro II should be prepared for venting in the following way:

Withdraw the probe.

Call up the tune page.

From the Gas menu ensure that all options are deselected.

Put the instrument into standby by deselecting operate.

Caution: Always close gas ballast valves before venting the instrument

The system can now be vented.

On the tune page select Other followed by Vent.

![Instrument Under Vacuum]

Select OK at the warning message.

The system now starts its automatic venting sequence.

Allow the system to vent automatically.

After the vacuum pumps are switched off, a few minutes will elapse until the automatic vent valves open. Shortly after this, the system will come up to atmospheric pressure and the source can be removed.

Caution: Venting the system by opening the vacuum lock valve is not recommended. Forcing the system to vent in this way can cause debris to be sucked into the analyser with a consequent loss of performance.
Removing the Ion Source

Instructions for API source removal are given later in this chapter. To remove a non-API source proceed as follows:

Withdraw the GC column (if fitted).
Disconnect the LC transfer line (if fitted).
Unplug the source connectors.
Disconnect all gas supply and pumping lines to the source.
Undo the two black thumb screws.
Pull the source out of the instrument.
Place the source on its stand and secure with the thumb screws provided.

Pumping Down

For API operation check that the source is correctly configured for the chosen mode of operation. (See “Changing Between Electrospray and APcI” in “Routine Procedures”.)

For non-API operation, insert the re-assembled ion source into the mass spectrometer.
Connect the GC column (if required).
Connect the LC transfer line (if required).
Connect the source connector plugs. (Failure to do so will cause MassLynx not to recognise the ion source in the mass spectrometer.)
Connect all gas supply and pumping lines to the source.

The API gas lines are connected simply by pushing the tubes into the coloured collars on the bulkhead connectors.

On the appropriate tune page select Other followed by Pump.
When vacuum has been achieved, select OPERATE and re-tune the instrument.
The API Source

Overview

The ionisation source is a robust assembly requiring little maintenance. Occasions when the inner source must be removed from the instrument include:

- When changing between electrospray and APcI operation.

  *It is necessary to remove the inner source to fit the appropriate counter electrode arrangement. See “Routine Procedures”.*

- When there is evidence of contamination.

  *A loss of sensitivity, not attributable to other causes, suggests that the counter electrode and sample cone may require cleaning.*

- When a better than normal reading is observed on the vacuum gauge(s).

  *This implies a blockage of the sample cone.*

- When, in APcI operation, source contamination leads to high voltage leakage across the discharge needle insulator.

Less frequently, it will be necessary to remove the source housing in order to remove and clean the skimmer and skimmer lens. This should be done under the following circumstances:

- When careful cleaning of the sample cone and counter electrode has failed to restore lost sensitivity.

- When charging effects are apparent.

  *Charging is evidenced by a noticeable progressive drop in signal intensity, often resulting in complete loss of signal. Switching to the opposite ion mode polarity, and back again, causes signal intensity to be momentarily restored.*

The RF (hexapole) lens, located between source and analyser, requires little maintenance. It is wise, following maintenance to the skimmer and skimmer lens, to direct a jet of nitrogen over the six rods to blow off any settled dust immediately prior to reassembly. After lengthy periods of use cleaning may be required. Symptoms are:

- Loss of sensitivity when quickly changing between masses, for example in multiple SIR mode.

- A slight loss of resolution.
The Counter Electrode

The counter electrode comprises two finely-machined metal blocks, each with four holes inclined to the centre line. When assembled the holes align to form four non-line-of-sight angled pathways.

The counter electrode is sometimes referred to as the “high voltage lens” or the “pepper pot”.

For early instruments, the blanking plug should be fitted for electrospray, while the discharge needle is essential for APcI operation.

Later instruments are equipped with separate complete assemblies for each mode.

The counter electrode and the back of the sample cone can be cleaned without venting the instrument.
Source Maintenance

The following instructions assume that the source is to be removed for maintenance purposes. When removing the source as part of the change-over to non-API operation, follow instead the instructions in “Routine Procedures”.

In this table the left hand column lists the sections in the following pages. The remaining columns indicate the steps to follow, in the correct order from top to bottom, for the various operations.

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<th>Cleaning the sample cone only</th>
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<tr>
<td>Removing the Counter Electrode</td>
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<td></td>
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<td>Removing the Sample Cone</td>
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<td>Removing the Source Housing</td>
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<td>Removing the Skimmer and Skimmer Lens</td>
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<td>Cleaning the Counter Electrode</td>
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<td>Cleaning Source Components</td>
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<td>Re-assembly</td>
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<td>✓</td>
<td>✓</td>
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</tbody>
</table>
Preparation for Full Source Clean

Deselect **OPERATE**.

Switch off the HPLC pump(s).

From the MassLynx tune window, select **Gas** and turn off the nitrogen flow.

Disconnect the electrical lead(s) from the probe. Withdraw the probe and lay it carefully to one side.

*It is not necessary to disconnect the probe’s sample line and gas supplies.*

Press **VENT**.

Disconnect the source’s main electrical lead (the “source high” lead) from the panel-mounted socket below the source.

If the source is configured for APcI, remove the coaxial connector from the socket on the source end flange.

Disconnect the drying gas tube (white collar) and exhaust tube (black) from the colour coded connectors above the flow control panel.

*To release a gas tube, press in the coloured collar on the connector and withdraw the tube.*
Removing the Inner Source

Release the two screws [3] and remove the end flange [4].

If appropriate, disconnect the sample cone voltage supply lead (purple) from the terminal pin [5]. Some sources are equipped with a spring loaded contact, and item 5 is absent.

Remove the two plastic-headed thumb screws [6] and withdraw the inner source assembly (complete with gas tubes, supply cable and O ring) from the stainless steel housing [7].

**Warning:** The inner source is heavy and may be hot. Handle with care, using the cable bracket and HT socket to hold the assembly during removal.
Removing the Counter Electrode

Remove the inner source as described above.

Remove the two locating screws [8] to release the counter electrode [9].

If the source is configured for APcI operation, disconnect the gold MRAC socket [10] as the counter electrode is withdrawn.

Separate the two component parts of the counter electrode, which are precisely aligned by a dowel and spigot.

If the APcI counter electrode is to be cleaned, separate the discharge needle (complete with insulator block) from the lens. Do not pull the electrical lead to release the pin, a leverage hole is provided in the insulator block to help free the pin if it is a tight fit.

Removing the Sample Cone

The sample cone should be removed when cleaning is necessary.

Remove the stainless steel housing [7] from the source housing by loosening the upper two of the four locating screws [2].

It is not necessary completely to remove these two screws. Unscrew each sufficiently to allow the housing to be withdrawn.

Remove the four screws [11] which secure the sample cone [12].

Withdraw the sample cone squarely from the metal housing to clear the locating dowel.

Remove the O ring [13] from the groove in the metal housing and store safely for reassembly.
In the above diagram the inner source and stainless steel housing have already been removed. However, if maintenance is to be performed on the skimmer, skimmer lens and lens stack alone, it is not necessary to remove these items.

Release the quick release clamp on the source housing vacuum line, collect the O ring seal and move the tube clear of the source housing, leaving the short metal connecting tube in place.

Support the source housing with one hand, while releasing the two black nuts with the other.

Pull the housing squarely away from the instrument.

The source housing extends approximately 6 centimetres into the analyser housing.

Collect the O ring seal.

**Caution:** To avoid contamination, the source opening should be covered whenever possible using the cover supplied for this purpose.
Removing the Skimmer and Skimmer Lens

With the source housing removed as described above proceed as follows:

Working through the front orifice, carefully withdraw the RF lens assembly sufficiently to allow the four leads to be disconnected. Remove the RF lens assembly.

_Caution:_ The skimmer is, of necessity, a delicate precision component requiring careful handling at all times.
Referring to the exploded diagram below:

Place the complete assembly on a bench, with the skimmer lens [6] uppermost.

Remove the terminal screw [1] and washer [2] to release the wire and solder tag [3].

Remove the four thumb nuts [4] and lift off the nylon washers [5].

Lift off the skimmer lens [6]. Lift off the four locating bushes [7] and remove the skimmer [8].

Take care to retain the insulating washers [9].

Cleaning the Counter Electrode

The counter electrode and the back of the sample cone can be cleaned, without venting the instrument, as follows:

Remove the counter electrode as described in the previous pages.

Using tweezers, wipe the back of the sample cone with a cotton swab soaked in a 50:50 mixture of methanol/water.
Clean the counter electrode plates and discharge needle assembly in a 50:50 mixture of acetonitrile:water or methanol:water in an ultrasonic bath.

A special tool, the micro inter-dental brush, is supplied to clean the holes in the lens.

**Cleaning Source Components**

Depending on the components removed:

Push a 0.2mm drill through the orifice in the centre of the sample cone to ensure that all particulate matter is removed.

*Ensure that the drill is no larger than 0.2mm, the nominal diameter of the orifice.*

Wipe off any excess contamination using water and a tissue.

Gently clean the sample cone, skimmer and skimmer lens using a swab wetted with concentrated formic acid, or by allowing concentrated formic acid to flow through their holes.

*Warning:* Formic acid is a hazardous substance and must be used only with extreme caution.

*Do not use any metal tool to remove deposits from the orifice in the skimmer.*

Brush all the peek insulator faces of the discharge needle assembly and the pin tip with a fibre glass pencil.

Immediately before re-assembly clean the sample cone, counter electrode plates, discharge needle assembly, skimmer, and skimmer lens in a 50:50 mixture of acetonitrile:water or methanol:water in an ultrasonic bath.

It is wise, following maintenance to the skimmer and skimmer lens, to direct a jet of nitrogen over the six rods of the RF lens assembly to blow off any settled dust immediately prior to reassembly. When further cleaning is necessary (see “Overview”) proceed as follows:

Immerse the complete assembly in methanol and place in an ultrasonic bath for 15 minutes.

Check that all screws are tight before replacing the assembly.
Re-assembly

The reassembly and refitting of the source follows the above procedures in reverse order. The following points should be noted.

- When refitting the locating bushes [7] to the holes in the skimmer [8] centralise each of the four studs in turn to allow the bushes to fall into place.

- Ensure that the counter electrode is correct for the technique to be used.

- When refitting the stainless steel housing, ensure that the heads of the four locating screws bear against the flats on the metal housing (not against the circular rim, as might occur if the inner source is not properly inserted). Adjust the four screws so that the inner source is roughly central in the housing, taking care not to over-tighten.

Once the inner source is replaced, pump down the mass spectrometer as described in “Routine Procedures”.

Probe Shaft Seals

The two ptfe-coated O rings which seal against the probe shaft can, if necessary, be replaced without otherwise disturbing the system. Old rings can be removed using the septum removal tool supplied. To prevent cracking, new O rings should be heated slightly in hot water before fitting. The inner source assembly must not be dismantled.
The EI/CI Ion Source

Filament Replacement

If the filament is to be removed as part of a more involved strip-down procedure, it is wise first to remove the source block assembly. Ignore the following instructions and proceed directly to “Disassembly of the EI/CI Ion Source”.

To remove the filament without further disturbing the source assembly:

Undo the two screws that secure the magnet assembly.

Remove the magnet assembly from the source block.

The filament can now be removed by undoing the mounting screw.
Disassembly of the EI/CI Ion Source

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Remove the four thumb nuts that hold the lens stack onto the source.

Lift the entire lens stack assembly vertically away from the source assembly.

Remove the three thumb screws that secure the source block assembly to the end flange assembly.

Withdraw the thermocouple connections from their respective sockets.

Gently lift the source block assembly off the mounting pillars.

Undo the four countersunk screws that retain the source top plate.

Remove the top plate, the aperture plate and the ion exit plate.

Undo the four screws that hold the magnet assemblies.

Remove both magnet assemblies from the source block.

Remove the filament by undoing the mounting screw.

Remove the trap assembly by undoing the screw that retains it.

Often further dismantling is unnecessary, proceed to “Quick Clean” Procedure. For extreme contamination Full Clean Procedure is recommended. Continue as follows:

Disassemble all the components of the source block assembly.

Undo the four thumb nuts and dismantle the lens stack assembly.
“Quick Clean” Procedure

In many cases it is sufficient to clean only the top surface of the repeller and the ion exit plate together with the trap, the aperture plate and the exposed surfaces of the first lens plate. When the source is cool:

Clean each of these items with micromesh or a fibreglass pencil.

Degrease with solvent

Blow out the source with a stream of dry nitrogen.

Full Clean Procedure

Before proceeding to clean the source components refer to the general guidelines in “Cleaning Materials” at the end of this chapter.

Identify the following components for cleaning:

- top plate
- ion exit plate
- repeller
- source block
- aperture plate
- trap
- support pillars
- lens plates

Clean each item with a fine abrasive (6000 or 12000 grade) or tungsten wool.

Degrease by washing with a suitable solvent.

Dry in an oven.

The aperture plate and ion exit plate must be treated with special care. Check for noticeable surface imperfections using a finger nail.

Before rebuilding the source, burnish the aperture plate, top plate and ion exit plate as follows:

**Caution:** Damage to moving parts may occur if this burnishing procedure is not followed.

Sprinkle molybdenum disulphide powder onto the parts and, using a piece of wood such as a pencil with the lead removed, rub the powder onto the mating surfaces and edges of the parts to give a thin grey coating.

Take particular care not to distort the sliding aperture.

Remove excess powder by blowing the parts.
Reassembly of the EI/CI Ion Source

The reassembly procedure is a reverse of the appropriate dismantling instructions. However, special care should be given to the following points:

- It is important to replace the magnet assemblies in the correct orientation. The two magnets should be fitted in a north-south configuration so that the two magnets attract each other. If the magnets are placed in a north-north or south-south orientation the electron beam within the ion source will not be collimated and the sensitivity will be greatly reduced.

- The lens plates each carry a notch. Ensure that these notches are aligned as shown.

- The lens stack assembly should be replaced on top of the source assembly and secured with its four retaining thumb nuts. Before tightening the thumb nuts, the source alignment tool should be inserted from the top of the lens stack to ensure that each of the lens plates and the ion exit plate of the ion source are concentrically aligned. Failure to align the lens plates will cause loss of instrument performance and sensitivity.

  Caution: The EI/CI source utilises an alignment tool with a blunt tip. The thermostrap source alignment tool (sharp tip) must not be inserted into an EI/CI source or the tool will be damaged.

- Before replacing the source, check the free movement of the sliding aperture plate.
The Thermospray Source

Replacement of the Sapphire Orifice Disk

The sapphire orifice disk may periodically become blocked with particulate matter. For reasons of clarity, the jet assembly is shown in the following diagram removed from the source. However, to remove and replace the disk it is not necessary completely to remove the jet assembly.

Vent the system and remove the source, as described earlier in this chapter.

Remove the protection bracket by undoing the two knurled retaining screws.

Remove the two clamps at the source end of the jet assembly by removing the two screws.

Remove the two screws which secure the ceramic block to the source block.
Remove the ceramic block, leaving the two connector blocks attached to the electrical wires.

Unscrew the grub screw sufficiently to free the vespel bush.

Gently withdraw the source end of the assembly from the hole in the source block.

*It is not necessary to undo the Valco nut or to disturb the clamps at the end-flange end of the assembly.*

Taking care not to twist the jet assembly, remove the retaining cap using the pliers provided.

Extract the sapphire disk.

It is not normally necessary to change the 24 carat gold seal when replacing the sapphire disk. However, in the event of leakage due to seal ageing, the seal should be replaced as follows:

Remove the old gold seal using a needle or small jewellers screwdriver.

Carefully fit the new gold seal into the recess as shown.
To replace the sapphire disk:

Lay the flat face of the disk on top of the gold seal.

Screw the retaining cap onto the disk until finger tight.

Rotate the cap a further 5° (approximately) using the pliers supplied. Do not overtighten.

To test the seal:

Hold the edge of a tissue at the end of the thread on the retaining cap.

Check that the tissue is dry.

Set the mobile flow rate to 1 ml/min.

Check that the tissue is still dry.

To reassemble the source follow in reverse order the above removal procedure, noting the following points:

Ensure that the base of the disk holder is located in the recess in the vespel bush. If necessary, gently pull the jet assembly while tightening the two clamps at the source end.

Ensure that the cut-out in the ceramic tube aligns with the ceramic block.

Removing the Jet Assembly

It is rarely necessary completely to remove the jet assembly. If this does become necessary proceed as follows:

Free the source end of the jet assembly as described above.

Loosen the two clamps at the end-flange end of the jet assembly by partially unscrewing the two screws.

Undo the Valco nut to release the end-flange end of the jet assembly.

Gently remove the complete jet assembly, including the protecting ceramic tube, from the source assembly.
Replacing the Jet Assembly

To refit the assembly follow the above instructions in reverse order. If the complete jet assembly is replaced, the resistance of the capillary should be checked with a digital volt meter set to \( \Omega \) before re-using the thermospray ion source.

*The source connector plugs should not be inserted when making this measurement.*

Touch the two meter probes together and note the reading.

Place one of the meter probes on the electrical contacts at the inlet end of the jet assembly.

Place the second probe on the electrical contact at the opposite end of the jet assembly. Note the reading.

The difference between the two readings should be between 1.1 and 1.4 ohms.

Insert the source connector plugs.

*With the system in vent*, set the capillary to room temperature using the potentiometer on the thermospray control unit.
Dismantling the Thermospray Ion Source

Thermospray ion sources generally require less maintenance and cleaning than EI/CI ion sources. When cleaning does become necessary proceed as follows:

Remove and dismantle the lens stack sub-assembly, as described earlier in this chapter for the EI/CI ion source.

Following the above instructions in “Replacement of the Sapphire Orifice Disk” free the source end of the jet assembly.

Remove the source block sub-assembly from the end flange sub-assembly.

Disassemble the source block as shown below.
Cleaning the Thermospray Ion Source

Before proceeding to clean the source components refer to the general guidelines in “Cleaning Materials” at the end of this chapter.

Identify the following components for cleaning:

- source block
- discharge electrode
- lens plates
- baffle
- repeller electrode

Clean each item with a fine abrasive or tungsten wool. (Only the internal surfaces of the source block need be cleaned.)

Degrease by washing with a suitable solvent.

Dry in an oven.
Reassembling the Thermospray Ion Source

The reassembly procedure is a reverse of the appropriate dismantling instructions. However, special care should be given to the following points:

- Insert the repeller electrode so that it comes up to the centre line of the source.
- Insert the discharge electrode below the centre line of the source. (If the discharge electrode is too high it will interfere with the spray from the jet.)

Fast Atom Bombardment

The Ion Source

The Dynamic-FAB ion source is a simple and open construction requiring little maintenance. Sputtered material and other contaminants can normally be removed without dismantling the source.

The Caesium Gun

Depending upon the amount of use and the operating conditions, the caesium pellet will eventually become depleted and will require replacement. To replace the pellet:

- Vent the instrument in the normal manner and remove the source top cover.
- Using a pencil, mark the position of the gun mounting bracket relative to the cover.
- Remove the gun from the cover by releasing the two screws.
- Loosen the barrel connector screw which secures the pellet's inner contact.
Remove the clamp plate by releasing the three securing screws and withdraw the caesium pellet.

Using the spent pellet as a model, carefully bend the contacts of the new pellet.

Fit the new pellet into the gun and refit the clamp plate, ensuring that the three outer contacts are securely held by the plate.

Locate the inner contact in the barrel connector, and tighten.

Refit the gun to the cover, using the pencil marks to ensure that alignment is restored.

Re-install the top cover and pump down the instrument.

When first using the source with a new pellet fitted, raise Anode in steps of 1kV up to 10kV.

*This will prevent sparking as the gun outgasses.*
The Electrospray Coaxial and Triaxial Probes

The Coaxial Probe

- 0.5 mm
- O Ring
- Lock Nut

The Triaxial Probe

- 0.5 mm
- 0.1 mm
- Make-up Flow
- Nebuliser Gas
Indications that maintenance is required to the electrospray probe include:

- An unstable ion beam.

Nebulising gas may be escaping from the sides of the probe tip arrangement.

Ensure that the lock nut is tightened onto the O ring.

The probe tip setting may be incorrect.

Adjust the sample capillary and, for the triaxial probe, the make-up flow capillaries as described in “Electrospray”.

- Excessive broadening of chromatogram peaks.

This may be due either to inappropriate chromatography conditions, or to large dead volumes in the transfer capillaries between the LC column and the probe tip.

Ensure that all connections at the injector, the column, the splitting device (if used) and the probe are correctly made.

- High LC pump back pressure.

This indicates a blockage in the solvent flow system. Samples containing particulate matter, or those of high concentrations, are most likely to cause blockages.

With no column in line, liquid flow rates should not generate back pressures in excess of 200 psi.

Check for blockages at the tube connections and couplings to the injector, the column and, if used, the flow splitter.

Use neat formic acid injections to clear blockages, rinsing thoroughly afterwards.

A blocked stainless steel sample capillary can often be cleared by removing it and reconnecting it in reverse orientation, “blowing out” the blockage.

- Gas flow problems.

To check that gas tube fittings have not been over tightened, release the fitting and use a clean LC syringe needle to ensure that no restriction is evident.

Remake the fitting finger-tight only.

To check that gas connections have been securely made, lightly place a finger over the tip of the probe. The nebulising gas flow meter indication should fall to zero.
The CE Probe

Indications that maintenance is required to the CE probe, in addition to those above relating to the coaxial probe, include:

- No sample peaks are observed when flushing through the CE capillary.
  
  *The sample is not being loaded onto the capillary due either to a low sample level or to a capillary blockage.*

- No sample peaks are observed when the CE voltage is applied.

  If there is no CE current:
  
  Check that there is a make-up solvent flow.

  Flush more sample through to clear possible air bubbles.

  Ensure that the probe is earthed.

  If there is a CE current:

  Check that the drying gas and nebuliser gas flow rates are not too high.

  Ensure that both ends of the CE capillary are at the same height.

- Unstable sample peaks are observed when the CE voltage is applied.

  Check that the probe tip is set up correctly.

  Check that there is a make-up solvent flow.

  Check that the source voltages, probe position and gas flows have been set up correctly.
The APcI Probe

Indications that maintenance to the APcI probe is required include:

- The probe tip assembly becomes contaminated, for example by involatile samples if the probe temperature is too low during operation (300°C).
- The appearance of chromatogram peak broadening or tailing.

Samples that give rise to a good chromatogram peak shape in APcI (for example reserpine and common pesticides) should display peak half widths of the order 0.1 minutes for 10 µl loop injections at a flow rate of 1 ml/min. The appearance of significant peak broadening or tailing with these compounds is most likely to be due to a broken fused silica capillary or probe tip heater assembly.

- Low LC pump back pressure.

For 50:50 acetonitrile:water at a flow rate of 1 ml/min, a LC pump back pressure less than 14 bar (200 psi) is indicative of a broken fused silica capillary or a leaking connector.

- High LC pump back pressure.

For 50:50 acetonitrile:water at a flow rate of 1 ml/min, a LC pump back pressure above 35 bar (500 psi) is indicative of a blockage or partial blockage in the fused silica capillary, in a LC connector or in the filter. It is advisable to change the inner filter pad (see “Replacing the Fused Silica Capillary” in the following pages) on a regular basis.

- Gas flow problems.

To check that gas tube fittings have not been over tightened, release the fitting and use a clean LC syringe needle to ensure that no restriction is evident. Remake the fitting finger-tight only.

Cleaning the Probe Tip

Remove any visible deposits on the inner wall of the probe tip with the interdental brush (supplied) soaked in methanol:water.

Before starting an analysis:

Insert the probe into the source.

Raise **APcI Probe Temp** to 650°C for around 1 minute with the nebuliser gas on, no mobile phase flow and a sheath gas flow of 100 l/hour.

*This procedure should remove any chemical contamination from the probe tip.*
To replace the probe tip heater:

Pull the probe tip assembly [items 1, 2 and 3] off the probe guide shaft [4].

Undo the two grub screws [1] on the outer stainless steel shroud [2] and withdraw the inner probe heater assembly [3]. If necessary, use a flat-bladed screwdriver to push the inner probe heater assembly out of the shroud (insert the screwdriver at the tip end of the assembly).

Reassemble the probe tip with a new heater assembly.
Replacing the Fused Silica Capillary

Remove the two screws [1] on the front face of the black plastic probe body [2] and expose the inner probe assembly.


Undo the two grub screws [5] that hold the LC filter [6] in the probe end plate and remove the filter/fused silica assembly from the back of the probe.

Cut a new length (280mm) of fused silica with a ceramic cutting tool ensuring that a square cut is obtained at each end.

Remove approximately 5mm of the polyimide coating from the probe tip end of the fused silica capillary.

*This is best done by rapidly “flashing” the end of the capillary with a flame and removing the remains of the coating with a tissue soaked in methanol.*

Remove the old capillary from the filter, and fit the new capillary using a new graphite/vespel ferrule.

Remove the probe tip assembly [7] and feed the new filter/capillary into the probe until the capillary protrudes by approximately 0.5mm from the 1/16” stainless steel capillary at the probe tip end.
Hold the capillary in this position and re-tighten the Swagelok nut at the first tee piece.

Lightly push the filter assembly against the capillary to avoid any dead volume.

Secure the filter into the probe end plate using the two grub screws and reconnect the probe tip assembly and probe body cover.
The Static-FAB Probe

*Warning:* Avoid touching the probe tip, as it may contain hazardous substances previously loaded onto the probe.

The only maintenance necessary is periodically to wash the tip in methanol, which can be done without dismantling the probe.

The Dynamic-FAB Probe

*Warning:* Avoid touching the probe tip, since it may contain hazardous substances previously loaded onto the probe.

The only maintenance necessary is periodically to wash the tip in methanol, which can be done without dismantling the probe, and to replace the mesh if blocked. To replace the mesh:

1. Unscrew the end cap and extract the old mesh.
2. Push a 5mm circle of 8µm stainless steel mesh into the recess in the end cap.
3. Screw the cap finger tight onto the tip until the mesh is firmly pressed against the dome.

*Caution:* Do not overtighten the end cap, otherwise the capillary may be restricted and the sintered wick may be damaged.
The Insertion Lock

The insertion (vacuum) lock is constructed around a 5/16” Hoke ball valve. After prolonged use the lock seals may become worn and in need of replacement. The ball seals and the inner ptfe seal have a long life provided that they are not over heated, or scored by debris. The outer O ring may need replacing from time to time.

Outer O Ring Replacement

If necessary, cool the solids insertion probe and remove it.

Remove the 3 screws [item 20] securing the probe support assembly [items 14-19 and 33-34] to the main body of the lock.

Remove the end cap [21], support liner [22] and outer O ring [13].

Remove the inner seal flange [12], O ring [11] and inner seal [10].

The inner seal and the support liner seldom require replacement but the opportunity should be taken to examine them. The support liner is not a vacuum seal and can therefore tolerate minor scratches.

Replace the outer O ring and, if necessary, the inner seal and support liner.

Reassemble the lock taking care correctly to orientate the assembly.
Shaft Seal and Ball Seal Replacement

Set **Source Temperature** to 40°C and allow the source to cool to below 100°C. If necessary, cool the solids insertion probe and remove it.

Disconnect the lock pumping line from the pumping port.

Vent the source region and remove the source in the normal manner.

Referring to the exploded diagram:

Remove the lock from the source end flange by removing the nut [item 38] and washer [39]. Take care not to lose the O ring [1].

Grip the body of the lock [9] in a vice and, using an adjustable spanner, unscrew the adapter flange [2]. Take care to retain the O ring [3].

Remove the shaft seal [4] and retaining ring [5].

Withdraw the ball seal [7] complete with O ring [6].

Rotate the handle [28] so that the slot in the ball [8]) is aligned with the axis of the lock enabling the ball to be withdrawn.

Prise off the handle cap [31], remove the retaining screw [30] and star washer [29], then lift off the handle together with the ptfe washer [27].

Push the spindle [23] into the lock, complete with O ring [26] and washers (24, 25), and remove.

The remaining ball seal [7] can now be removed.

Fit a new ball seal into the body of the lock.

Fit the washers and a new O ring to the spindle and, from inside the lock, insert the spindle.

Refit the ptfe washer, handle, screw and cap.

Clean the ball with solvent and a soft tissue. Check that it is not scored or damaged in any way. Rotate the handle so as to enable the ball to be replaced.

Fit a new ball seal and O ring. Replace the retaining ring and fit a new shaft seal. Fit a new O ring to the nut and screw the nut to the valve body. Tighten gently with the adjustable spanner.

Refit the lock to the source end flange, refit the source and reconnect the lock pumping line.
The Particle Beam Interface

The particle beam interface is an inherently simple device with few moving parts and therefore requires little maintenance.

Cleaning the Nozzle and Skimmers

With long periods of operation there can be a build up of sample or buffer salts on the skimmers or the nozzle at the end of the desolvation chamber. This can eventually lead to a blockage of these apertures, usually at the first skimmer, which shows itself as a complete loss of sensitivity. Also, the source pressure will not be affected by the flow of helium and solvent into the desolvation chamber.

Access to the nozzle and first skimmer can be gained by removing the desolvation chamber from the momentum separator housing. Access to the second skimmer is gained by removing the flange on the back of the momentum separator housing.

Caution: These components are precision engineered and must be treated with care.

Any deposits can be removed by a clean lint-free tissue and a suitable solvent, taking care not to leave behind any fluff that may cause a second blockage. If more severe cleaning is required, the components can be cleaned in an ultrasonic bath of a suitable solvent. In the unlikely event of it being necessary to clean the nebuliser, it can be cleaned in an ultrasonic bath. Remove the outer cap from the nebuliser body and place them both in the ultrasonic bath in a suitable solvent. The nebuliser should not spend more than ten minutes in the ultrasonic bath.
**The Analyser**

The analyser element of any high performance quadrupole mass spectrometer is, of necessity, a precisely machined and aligned assembly.

Quattro II is fitted with prefilter assemblies that are designed to protect the main analysers by absorbing the majority of any contamination. The prefilters are not as mechanically critical as the main rods, as they have only RF applied and are therefore not resolving. They do, however, act as a high pass filter, and will reject ions of low mass before they enter the main rods.

MS1 is equipped with two prefilters, and it may periodically become necessary to remove the first of these, the entrance prefilter, for cleaning.

The need to clean the prefilter rods is usually indicated by poor peak shape or loss of resolution, although other more likely causes, such as contamination of the source or of lens 4 (the differential aperture which, for non-API sources, remains in the housing) should be eliminated first. Any charging which may occur will be more pronounced under CI conditions, when the low mass peaks are particularly strong.

*Caution:* Cleaning the analyser on site is not a task to be undertaken lightly. It should be done only when there is clear evidence that the analyser is the source of lack of performance. The main rods of the analyser are precision ground, as are the ceramics in which they are mounted. Assembly is also precision work, and therefore under no circumstances should the analyser rods be removed from the analyser assembly.

It is unlikely that there will be any means on site for measuring the assembly to the micron level required. If analyser charging effects cannot be resolved by the techniques below, it is almost certain that the analyser will have to be returned to Micromass for refurbishment.
Removing the Entrance Prefilter

Having vented the system, as described at the beginning of this chapter, and removed the analyser housing top plate proceed as follows:

Unscrew the grub screw from the castellated ring. Slide the castellated ring towards the front of the instrument to clear the MS2 analyser.

Slide the MS2 analyser toward the rear of the instrument to its furthest extent (3mm).

Pull the two CID gas tubes off the collision cell. Remove the two gold MRAC connectors from the Altrincham lens, and disconnect the 9-way socket.

Release the four copper leads from the feedthroughs adjacent to MS1, by removing the cheese head screws and washers.

Disconnect the two barrel connectors and release the entrance prefilter connecting wires from the barrel connectors.

Free the MS1 analyser in the housing by loosening the four thumb screws sufficiently to allow them to swing clear of the mounting brackets. Free the entrance prefilter housing by undoing the two socket screws in a similar way.
Using the second prefilter and post-filter studs, *carefully* lift the MS1 analyser, complete with collision cell, away from the housing leaving the entrance prefilter in position. It will be necessary to manoeuvre the analyser slightly as it is lifted, in order to clear the MS2 analyser and the entrance prefilter.

Carefully lift the entrance prefilter assembly out of the analyser housing, manoeuvring the assembly slightly to clear the entrance ring.

Cover the analyser housing while the analyser is removed.
Dismantling and Cleaning the Entrance Prefilter

**Caution:** To maintain the correct location of the prefilter rods on re-assembly, it is essential that only one prefilter rod at a time is removed for cleaning.

Remove the locating screw and separate the entrance prefilter housing from its mounting bracket.

Undo and remove the outer nuts trapping the upper connecting wires to the prefilter rods, the copper spacers and the threaded rod. Remove the two connecting wires, noting their positions to help reassembly.

Place lint-free paper in between the prefilter rods. (This prevents damage when a rod is released.)

While holding one prefilter rod unscrew the inner nut from its terminal, remove the spacer and unscrew the stud.
Remove the prefilter rod from the assembly.

Remove the ion burn using Polaris powder, or 6000 grade micromesh.

Clean the rod in a solvent to remove grease. Use an ultrasonic bath if possible.

Loosely refit the prefilter rod, stud, spacer and inner nut into the assembly.

Align the ends of the rods by setting the assembly on a clean flat surface with the rods vertical, and tightening the inner nut.

Repeat the above procedure for each of the remaining prefilter rods in turn.

**Cleaning the Main Analyser Assemblies**

As stated above, the main rods *must not be disassembled.*

Having removed the MS1 assembly as described above, separate the main MS1 analyser assembly from the entrance prefilter and from the collision cell.

Roll up a narrow strip of absorbent lint-free paper, longer than the analyser assembly. One end should be screwed up tightly to allow the strip to be passed through the prefilter, analyser and post filter.

Use a solvent such as methanol to wet the paper and move the strip up and down between the analyser rods.

Remove the strip and use dry nitrogen gas from a cylinder (*not* compressed air) to blow out any dust or particles.

Release and remove the MS2 analyser assembly from the housing, in a similar fashion to the MS1 removal procedure.

Repeat the cleaning procedure for the second analyser, using a clean paper strip.

**Replacing the Analysers**

Reassembly is the reverse of the appropriate dismantling procedure. Take extra care to ensure that all electrical connections are correctly and securely made, and that the various mechanical assemblies are accurately aligned within the housing on their locating dowels.
The Detector

Quattro II’s detector systems have been designed for trouble-free operation over many years. The sealed photomultipliers are safe from contamination and pressure surges (the traditional enemies of mass spectrometer multipliers) and the conversion dynodes are tolerant of ion burn. The phosphors have also proved to be reliable and long-lasting. No routine maintenance is therefore required.

> It is strongly recommended that assistance is sought from Micromass if maintenance to the detector system is thought necessary due to “spikes” or unacceptably high noise levels.

The Vacuum System

Pirani Gauges

The pirani gauge heads do not require routine maintenance.

Active Inverted Magnetron Gauges

For information on cleaning the active inverted magnetron gauges, refer to the Edwards literature supplied with the instrument.

Foreline Trap

The foreline trap is used to stop oil vapour migrating from the rotary pump to the mass spectrometer. During normal use, the activated alumina will absorb any oil vapour. The activated alumina becomes brown in colour after absorbing oil, and should be changed when the discolouring reaches the region of the trap furthest from the pump (the vacuum side).

> With the instrument vented and the pump switched off, replace the sorbent as described in the manufacturer's literature.

Turbomolecular Pumps

The turbo pump oil reservoir should be changed annually. Refer to the manufacturer's literature for details.
Rotary Pumps

Gas Ballasting

When rotary pumps are used to pump away large quantities of vapours - for example CI reagent gases and HPLC effluents - the solvent or gas tends to dissolve in the vacuum oil which usually leads to an increase in the backing line pressure. Gas ballasting is a method used to purge the oil of the contaminants.

Gas ballasting, which should be carried out whenever the backing pressures are high or immediately after a long period of CI or LC operation, is performed by opening the gas ballast valve on top of the rotary pump.

*In particular, take care when using ammonia as a CI reagent gas that the oil in the backing pumps is not wet from previous LC operation. Rapid deterioration of the rotary pumps will occur under these conditions.*

- Avoid venting the instrument when the rotary pumps are gas ballasting.
- Do not gas ballast rotary pumps for more than 2 hours under any circumstances.
**Rotary Pump Oil**

The oil in the rotary pumps should be maintained at the correct level at all times. Check the oil levels at weekly intervals.

The oil in the rotary pumps should be changed after 3000 hours operation (approximately quarterly). It will be found easier to drain the oil while the pump is still warm.

Vent and shut down the instrument.

Drain the oil through the drain hole situated near the oil level sight glass.

Replace the drain plug and refill the pump with the correct grade oil to the correct level.

*For LC systems equipped with Edwards rotary pumps, TW oil must be used for the LC inlet pump(s) and the source backing pump.*

For further servicing information concerning the rotary pumps refer to the manufacturer’s manual.

**“Hot Oil” Single Stage Rotary Pumps**

The particle beam / thermospray rough pumping system includes a high temperature “hot oil” single stage rotary pump. Pumps of this type run at relatively high temperatures, thus preventing water and eluent vapours from condensing in the pump.

After switching on a hot oil pump, allow about 30 minutes for the pump to reach operating temperature before using the particle beam or thermospray system.

It is important regularly to monitor the condition of the oil, which must be changed as soon as it becomes noticeably discoloured. This would typically be at intervals of 2-6 months.

It is important to drain the oil from the system while it is still hot. Immediately the pump is switched off all the oil should be drained out. The pump should then be partially refilled with clean oil, flushed and drained. This flushing procedure should be repeated two or three times before refilling the pump with fresh oil.
Cooling Fans and Filters

Always ensure that none of the cooling fans is obstructed. It is essential that the filters are checked at regular intervals, and replaced if there is any doubt about their effectiveness. Simply remove the retaining screws, remove the cover and withdraw the filter.

Cleaning Materials

It is important when cleaning sensitive components to maintain the quality of the surface finish. Deep scratches or pits can cause loss of performance. Fine abrasives should be used to remove dirt from metal components. Recommended abrasives are:

- 1200 grade emery paper.
- 6000 grade micro-mesh.
- Tungsten wool.
- Fibre glass pencil (EI/CI source “quick clean” only).

After cleaning with abrasives it is necessary to wash all metal components in suitable solvents to remove all traces of grease, oil and, if micro-mesh is used, rubber. The recommended procedure is to sonicate the components in a clean beaker of solvent and subsequently to blot them dry with lint free tissue.

Recommended solvents are:

- Isopropyl Alcohol (IPA).
- Ethanol.
- Acetone.
- Methanol
- Acetonitrile

Finally, components should be completely dried in an oven prior to re-use.
Preventive Maintenance Check List

✖ Avoid venting the instrument when the rotary pumps are gas ballasting.

✖ Do not gas ballast rotary pumps for more than 2 hours under any circumstances.

For full details of the following procedures, consult the relevant sections of this chapter and/or refer to the manufacturer's literature.

Daily

• Gas ballast the main rotary pump lightly for 20 minutes at the end of a day's electrospray, CI or dynamic FAB operation.

• Gas ballast the main rotary pump for 30 minutes at the end of a day's megaflow or APcI operation.

• Gas ballast the first stage particle beam/thermospray pump during operation.

• Gas ballast the main rotary pump for 30 minutes immediately after particle beam operation.

Weekly

• Gas ballast the main rotary pump lightly for 30 minutes following a week's EI or static-FAB operation.

• Check the rotary pump oil level and colour.

   Gas ballast lightly for 30 to 60 minutes both before and after topping up the oil.

• Check the water chiller level and temperature (if fitted).

Monthly

• Check the foreline trap material for colour.

• Check all cooling fans and filters.

• Check the cleanliness of the active inverted magnetron gauge(s).

Four Monthly

• Change the oil in the rotary pump(s) after 3000 hours operation.

   Gas ballast lightly for 30 to 60 minutes both before and after changing oil.

Annually

Change the turbomolecular pump oil reservoirs.
Fault Finding

Introduction

The majority of faults that occur can be traced to a malfunction of the ion source or inlet system. On systems equipped with more than one ionisation source, this can often be confirmed by changing sources to see if the fault “moves” with the source. Should a fault occur soon after a part of the system has been repaired or otherwise disturbed, it is advisable first of all to ensure that this part has been correctly refitted and/or adjusted and that adjacent components have not been inadvertently disturbed.

The API Source

No Beam

Refer to the relevant chapters of this manual and check that:

- Solvent is reaching the probe tip and the solvent flow rate is as required.

  *For solvent flow rates below 100 µl/min it may be necessary temporarily to turn off the nebulising gas and remove the probe tip to allow the solvent to be seen.*

- The flow of drying gas, nebuliser gas and, for APcI, sheath gas is on.

- The off axis adjuster is not completely off axis.

Unsteady Beam

Refer to the relevant chapters of this manual and check that:

- The probe voltage (electrospray) and counter electrode are tuned correctly.

- The capillary is not protruding too far from the end of the probe.

- The probe is not too far into the source.

- The flow of solvent from the HPLC pump is correct and steady.

  *To do this, remove the probe, degas the solvent, increase the flow rate for several minutes to purge any trapped air then reset and remeasure the flow rate.*

- Solvents used have been sufficiently degassed.

- The nitrogen flow of drying gas, nebuliser gas and, for APcI, sheath gas is steady. The nitrogen supply pressure should be 7 bar (100 psi) ±10%.

Should the preceding checks fail to reveal the cause of the problem:

- Observing the readbacks, check that **Cone** varies between 0 and 200V, and that **Skimmer Lens Offset** is variable between -50V and +50V.
Low Intensity Solvent Ions

Refer to the relevant chapters of this manual and check that:

- The Source and MS parameters are optimised.
- The probe position in the source is optimised.
- The solvent flow rate is not too high.
- Nitrogen flow rates are optimised.
- The off axis adjuster is optimised.
- The source is clean.

High Back Pressure

For electrospray, a higher than normal back pressure readout on the HPLC pump, together with a slowing of the actual solvent flow at the probe tip, can imply that there is a blockage in the capillary transfer line or injection loop due to particulate matter from the sample. To clear the blockage:

Remove the probe from the source and increase the solvent flow to 50 µl/min to remove the blockage.

Often, injections of neat formic acid help to redissolve any solute which has precipitated out of solution.

If the blockage cannot be cleared in this fashion:

Remove the finger-tight nut and tubing from the back of the probe.

If the back pressure remains high, replace the tubing with new tube (or first try removing both ends of the tube).

If the back pressure falls, replace the stainless steel sample tube inside the probe (or try reversing the tube to blow out any blockage).

Reconnect the tubing to the probe.

The solvent flow can be readjusted and the probe replaced into the source.

To check the flow rate from the solvent delivery system, fill a syringe barrel or a graduated glass capillary with the liquid emerging from the probe tip, and time a known volume, say 10µl.

Once the rate has been measured and set, a note should be made of the back pressure readout on the pump, as fluctuation of this reading can indicate problems with the solvent flow.
For APcI a higher than normal back pressure readout on the HPLC pump can imply that, after a long period of use, the filter pad requires replacement.

**Low Pressure**

An “improved” reading on the analyser and backing vacuum gauges can mean that the sample cone has become blocked, or partially blocked, and the source requires cleaning.

**The EI/CI Ion Source**

Faults on EI/CI ion sources are often associated with the filament. Most other faults are caused by contamination and usually give rise to instability in the ion beam and/or loss of sensitivity.

The following information refers to the EI/CI source operating in the EI mode. filament current, trap current, emission (source) current and source temperature can all be monitored on the EI tune page.
No Source Current

The source current is the flow of electrons between the filament and the ion chamber.

Zero source current indicates that the filament may be open circuit or shorted to the shield.

No Source Current, Zero Filament Current

If the filament is open circuit, the filament current will be zero. To confirm an open circuit filament condition:

Switch to Standby (OPERATE deselected).

Remove the connecting plug on the end-flange.

Use a multimeter on Ω range to check for continuity between pins 2 and 4 on the smaller (7 way) source connection socket.

The fault may be a burned out filament or a loose connection. The source must be removed before further checks can be made.

No Source Current, High Filament Current

If the filament is short circuited by the shield then the filament current will read full scale.

This condition may be intermittent, the short circuit only occurring when the filament is hot or when high emission currents are selected.

High Source Current

In a correctly functioning source operating in EI mode the source current should be approximately 5 to 10 times the emission (trap) current.

High source current, even at low emission current settings, is usually indicative of a short circuit or leakage path between the filament and the ion chamber. The general remedy is to fit a new filament, following the instructions in “Routine Maintenance”. If the fault persists:

Check that the source is not dirty.

Check that the electron energy is the value demanded (normally 70 volts).

The electron energy is the voltage difference between the filament and the ion chamber. An electron energy reading that is zero or significantly lower than the demanded value indicates a leakage from the filament to the ion chamber or to ground.
If the electron energy is low:

Check that the filament shield is clear of the magnet cap.

Check all leads for possible short circuits or leakages

**Incorrect Emission (Trap) Current**

*The emission current, in EI mode, is the current generated by the flow of electrons between the filament and the trap. Filament current regulation is by feedback of the trap current.*

**Low or unstable trap currents** are usually caused by:

- An insulating layer on the trap surface. In this case the symptom will be accompanied by a high filament current.
- A filament that has aged.
- An obstruction in the ion source, such as a probe tip or a piece of broken column.

**Full scale trap current** at all settings usually indicates a short circuit between the trap and ion chamber.

If problems arise after cleaning and refitting the ion source, then the probable causes are:

- The filament is out of alignment
- The source magnets are repelling rather than attracting each other.
- An electrical connection has been omitted or incorrectly made.
- One or both of the source magnets are weak.

In each case, the ion source must be removed (see “Routine Maintenance”) and the appropriate action taken.
**Dynamic-FAB**

*Warning:* Avoid touching a used wick, since it may contain hazardous substances previously loaded onto the probe.

Poor performance in the dynamic-FAB mode is often due to sample delivery problems. To locate and rectify the problem:

Undo the capillary connector at the injector end (port C). Check that the end is clean, cut square and correctly seated in the ferrule and connector port. Retighten the connector.

Inspect the remaining injector and tubing connections for possible dead volume problems. The two Valco connectors should be adequately tightened such that no sliding movement occurs in the ptfe ferrules.

Refill the mobile phase vial with approximately 0.5ml of solvent. Ensure that the assembly is gas tight when sealed.

Unscrew the back end of the injector.

Using needle-nosed pliers or tweezers, remove the rotor and inspect the four injection volume indents and the polymer surface for damage.

Clean the rotor in an appropriate solvent and dry with compressed air.

Reassemble the injector.

Pressurise the vial to approximately 4 bar (60 psi) and check that a flow of nominally 5 µl/min is observed at the probe tip.
The Gas Chromatograph

Many of the problems encountered are readily solved by attention to detail. Listed below are a number of common faults which can occur.

Low Sensitivity

- Split valve flow rate set too high.
- Septum purge flow rate set too high.
- Injector/column connecting nut leaking.
- Column broken below the split vent.
- A leak at the connection to the interface line.

Poor Resolution and Peak Shape

- Column broken below the split vent.
- Cold spot in the interface caused by a failed heater, or by cool air entering the interface.
- Dead volumes.
- Graphite or silicone rubber in the system.
- Dirty injector volume.
- Column pushed too far up the injector so that the needle is injecting past the end of the column.
- Column flow rate too slow or fast.
Vacuum System

The performance of any mass spectrometer is severely impaired by the lack of a good vacuum, particularly in the analyser region.

At analyser pressures above $10^{-4}$ mbar there will be a general loss of resolution and an increase in background noise. As the pressure increases further, the air peaks will become large unresolved humps and the high voltage on the photomultipliers may set up gas discharge that will reduce to zero the gain of the multiplier.

Poor Vacuum

A high pressure reading on the source or analyser pirani gauge will cause the corresponding turbomolecular pump to switch off with a resulting loss in fine pumping. Before suspecting a fault or a vacuum leak (unless the system has recently been disturbed) check the following:

- A dirty active inverted magnetron gauge will indicate a poor vacuum. Check the condition of the gauge.
- Follow the gas ballasting procedure detailed in the “Routine Maintenance” section.

A turbomolecular pump will not operate satisfactorily if its rotary backing pump has failed. See the manufacturer’s manuals for details on maintaining the pumps.
Vacuum Leaks

It is most unusual for flanges that have not been disturbed to leak. Most leaks occur on the inlet systems and ceramic feedthroughs.

If leaks are suspected proceed as follows:

Spray the suspect area with iso-propyl alcohol while monitoring the appropriate vacuum reading.

*Warning:* Care should be exercised to minimise the fire risk when using flammable solvents.

If a leak exists it will result in a rise in pressure as the solvent enters the system.

*It is recommended that a stand-alone vacuum gauge, connected to the appropriate head, is used for leak checking. This will give a quicker response than the vacuum readouts.*

Patient use of this technique can usually pinpoint a leak. If the leak is small and in the source or inlet region, then helium or argon can be used as a search gas, as follows:

Fit the EI source and, operating in the EI+ mode, tune to the appropriate mass ($^{m/z} 4$ for helium, 40 for argon).

Connect a length of flexible tubing to the gas cylinder outlet, and direct the flow of gas around the suspect area.

Monitor the intensity of the peak. The peak intensity increases as the search gas enters the system.

*To pinpoint very small leaks use a nozzle to produce a narrower jet of gas.*

Leaks on flanges can usually be cured by tightening the flange bolts or by replacing the seal. Similarly, couplings on interface lines can be sealed by tightening the nuts or by replacing the ferrules.

If a leak on a feed-through is found then the unit should be replaced, or returned to Micromass for repair, as soon as convenient.

*A temporary cure can sometimes be effected using sealants produced for this purpose, epoxy glues or even nail varnish. However this can lead to trapped volumes, preventing the ultimate vacuum being achieved and perhaps causing pressure pulsing.*
Electronics

Supplied with Quattro II is a set of schematic diagrams and wiring information, provided for users wishing to attempt their own repairs or modifications. However, it is strongly recommended that any such work is entrusted only to factory-trained engineers.

To locate faults a good quality digital multimeter is required, having a suitable voltage range. Voltages up to 10kV may have to be measured so a high voltage probe should also be available.

*Warning:* There are high voltages present throughout the mass spectrometer. Extreme caution should be taken when taking measurements with a meter or an oscilloscope. In the STANDBY mode (OPERATE deselected) the high voltages are switched off in the source and analyser assemblies, but high DC voltages and mains voltages remain in the bench.

*Caution:* Quattro II’s electronic systems contain sophisticated but extremely sensitive components. Any fault finding procedures should be carried out only by skilled personnel observing the most stringent precautions against electrostatic discharge.

Ripple

Peaks appear to vary cyclically in intensity when there is ripple superimposed on the peak. Possible causes are:

- Unstable filament current (EI/CI source).
- A source heater short circuited to the ion chamber.
- Unstable power supplies in the source supplies or the RF/DC generators.
- Unstable photomultiplier supply.
- Vibration from the rotary pumps, GC fan, or even other equipment in the same building.

The frequency of the ripple, measured using an oscilloscope, can often help locate the source. Mains frequency ripple, for example, points towards an unstable power supply or vibration from mains powered machinery.
Loss of Sensitivity

As the ion source becomes dirty after prolonged use, the performance will degrade. Unstable or reduced ion currents, high ion energy and high repeller values are indicators that the source needs cleaning. The usual remedy is to remove and refurbish the ion source as described in “Routine Maintenance”.

However there are many other conditions that can cause the loss or reduction of the ion beam. The following check list should help in locating the cause of the problem. Also refer to the relevant following sections of this chapter for information specific to loss of sensitivity in the various ionisation modes.

- Check that all the circuit breakers are ON.
- Check that the analyser pressure is better than $10^{-5}$ mbar.
- Check that the EI/CI aperture plate is not partially or totally closed.

If all seems well, then proceed as follows with the EI/CI source fitted:

- Check that the ion energy is correct and in agreement with the tune page setting. In positive ion mode the ion energy should be a negative voltage.
- Check that the source voltages are present and vary with the tune page settings.
- Set the display gain to 500, remove the pre-amplifier cover and touch the input lead. Increased noise levels should be observed on the baseline if the amplifier circuits are working correctly.
- Check the lens voltage readbacks.

**Warning:** Dangerous voltages exist in the analyser region and due care is needed when taking measurements. Always deselect OPERATE before connecting or disconnecting any measuring equipment.

- Check the DC voltages to the analysers, supplied by the RF/DC control circuits.

  On the tune page, set M1 to 2000 and M2 to 0.

  The measured DC voltage should be about 475 volts. The majority of failures results in either a voltage of 0 or 1000V being measured.

Further investigation in the RF generator should be made only by a competent electronics engineer, experienced in RF circuits. If assistance is required, please contact Micromass.
High Noise Levels

Quattro II’s detector systems have been designed for trouble-free operation over many years. The sealed photomultipliers are safe from contamination and pressure surges (the traditional enemies of mass spectrometer multipliers) and the conversion dynodes are tolerant of ion burn. The phosphors have also proved reliable and long-lasting.

In the event of unacceptably high noise levels or “spikes”, and when electronic faults have been eliminated, proceed as follows:

With **deselect**, disconnect the phosphor coaxial connector on the detector end flange and earth the central terminal on the end flange socket.

Select **operate**. A significant reduction in the noise level indicates electrical breakdown in the phosphor.

Deselect **operate** and replace the connector. Repeat the above procedure for the two other high voltage connectors.

Because of it’s cost, replacing detector components should not be regarded as part of a fault finding procedure. It is strongly recommended that assistance is sought from Micromass if maintenance to the detector system is thought necessary.
Fault Finding Check List

**Warning:** There are high voltages present throughout the mass spectrometer. Extreme caution should be taken when taking measurements with a meter or an oscilloscope. In the STANDBY mode the high voltages are switched off in the source and analyser assemblies, but high DC voltages and mains voltages remain in the power supply units. Any investigation in the RF generator must be made only by a competent electronics engineer, experienced in RF circuits. If assistance is required, please contact Micromass.

General Loss of Performance

**No Beam**

Refer to the relevant chapters of this manual and check the following:

- Normal tuning parameters are set and, where appropriate, readback values are acceptable.
- All necessary cables have been correctly attached to the source and probe.
- OPERATE is on (see the front panel).
- The source has been assembled correctly and is clean.
- Set the display gain to 500, disconnect the detector head amplifier and touch the central pin of its coaxial connector. Increased noise levels should be observed on the baseline if the amplifier circuits are working correctly.
- Check the DC voltages to the analyser, supplied by the RF/DC control circuits.
The flow chart opposite is included to assist in the location of faults in the event of a complete loss of the ion beam. The chart, with the accompanying list of operating parameters, refers to the Quattro II in the EI positive ion mode. The chart should be used in conjunction with sections “The EI/CI Ion Source” and “Loss of Sensitivity”.

For other modes (or if the fault is thought not to be associated with the source) the chart may be used in conjunction with other sources, by-passing the source-related checks.

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<td>Filament Current</td>
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<td>DC Generator Voltage</td>
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*These parameters are approximate only and are for an instrument in a good state of repair. Source parameters in particular vary with operating conditions and contamination levels.*

**Unsteady or Low Intensity Beam**

Should the preceding checks fail to reveal the cause of the problem check that:

- Gas and liquid flows are normal.
- The analyser pressure is better than 1x10⁻⁴ mbar.
- The prefilter is not heavily contaminated.
- Source and probe voltages are present and vary with the tune page settings.
The above chart shows a logical fault finding procedure for a system failing to show peaks in the EI positive ion MS mode, in OPERATE with reference gas on and with adequate vacuum levels.
Reference Information

Most samples can be purchased from:

The Sigma Chemical Company Ltd.
Fancy Road, Poole, Dorset.
BH17 7NH
United Kingdom.

Tel: (UK) 01202 733114
(International) +44 1202 733114

Positive Ion API

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## Horse Heart Myoglobin

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Molecular Weight: 16951.48  

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## Polyethylene Glycol

**PEG + H\(^+\)**  
Reference File: PEGH

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Reference Information  
Page 222
### PEG + Na⁺

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### Sodium Iodide and Caesium Iodide Mixture

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### Horse Heart Myoglobin

Reference File: MYONEG

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<th>1129.091</th>
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<td>1411.615</td>
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### Mixture of Sugars

Reference File: SUGNEG

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<th>503.161</th>
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<tr>
<td></td>
<td>665.214</td>
<td>827.267</td>
<td>989.320</td>
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Reference Information
Page 224
The following reference materials are recommended for tuning and calibration purposes over the indicated mass ranges in the EI positive ion mode. To include the air peaks in the calibration process, check the box on the Calibrate dialog window.

<table>
<thead>
<tr>
<th>Ref. File Name</th>
<th>Chemical Name</th>
<th>Inlet System</th>
<th>Mass Range</th>
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<tr>
<td>HEPTACOS</td>
<td>Heptacosfluorotributylamine (FC 43) [H5262]</td>
<td>Reference Inlet</td>
<td>50-600</td>
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<tr>
<td>ULTRAM</td>
<td>Ultramark</td>
<td>Solids Probe</td>
<td>50-2000</td>
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**Air**

Reference File: AIR

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Heptacosa (perfluorotributylamine) (FC43)

Reference File: HEPTACOS

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<th>Calculated m/z Value</th>
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<td>130.99199</td>
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# Ultramark

Reference File: ULTRAM

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<th>Calculated m/z Value</th>
<th>Relative Intensity (% base peak)</th>
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<td>168.989</td>
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<td>0.063</td>
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<tr>
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Fast Atom Bombardment

Caesium Iodide and Rubidium Iodide Mixture

Reference File: CSRBI

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<th>m/z</th>
<th>Intensity (%)</th>
<th>m/z</th>
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The masses and intensities for caesium iodide alone (reference file CSI) are listed in “Fast Atom Bombardment”